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FROM THE
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VOLUME 2

1912-1913

MINNEAPOLIS
Published by the University of Minnesota
1913

JAN 29 1917

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Johnston, John B. Upon the morphology of the forebrain in fishes, Anatomischer Anzeiger, Vol. 40, 1912, p. 531-540, 6 figures.

*The telencephalon in Cyclostomes, Journal of Comparative Neurology, Vol. 22, 1912, p. 1-70, 41 figures.

*On the teleostean forebrain, Anatomical Record, Vol. 6, 1912, p. 423-438, 3 figures.

*Nervus terminalis in reptiles and mammals, Journal of Comparative Neurology, Vol. 23, 1913, p. 97-120, 12 figures.

*The morphology of the septum, hippocampus, and pallial commissures in reptiles and mammals, Journal of Comparative Neurology, Vol. 23, 1913, p. 371-478, 93 figures.

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THE INTERSTITIAL GRANULES (LIPOSOMES) IN FATTY METAMORPHOSIS OF STRIATED MUSCLE.[1]

By E. T. Bell, *Institute of Anatomy, University of Minnesota.*

(Plate XVIII.)

It has been shown in previous papers (Bell, 1910[6], 1911[7]) that many normal tissues contain numerous droplets of a fatty nature that are seldom seen in the ordinary examinations for fat.

When a section of fresh tissue is cleared with potassium hydrate (1 to 5 per cent.), a number of small, more or less refractive droplets are usually visible. These are the liposomes. Some are strongly refractive, others are faintly refractive; and all intermediate forms between these two extremes may be present. They may be seen when the fresh tissue is examined in aqueous humour or in the tissue juices, but not so distinctly as in specimens cleared by potassium hydrate. All of the liposomes are readily soluble in absolute alcohol and ether. The extent to which they are shown in examinations for fat depends upon the character of the fat stain employed and the previous treatment of the tissue.

The strongly refractive liposomes may be easily stained with ordinary fat stains, and, as a rule, are only slightly affected by fixatives. These are commonly regarded as droplets of neutral fat.

The faintly refractive liposomes, however, are more difficult to demonstrate. In the ordinary examinations for fat these droplets are either entirely unstained or so faintly stained that their fatty nature is not suspected. They disappear rapidly as a result of post-mortem changes, and in fixing reagents. They may be stained in fresh tissues with Herxheimer's scarlet red or with especially strong simple alcoholic solutions of scarlet red or sudan III.

Frequently a large majority of the liposomes, especially those in muscle, are of intermediate refractive power. These may stain a pale brown with osmic acid, but they are often not coloured at all by this reagent. Herxheimer's solution shows these droplets clearly, but less intensely stained than the strongly refractive droplets. The liposomes of this class are clearly stained by strong simple alcoholic solutions of scarlet-red or sudan III., but if the stain be weak they may not be shown. A simple alcoholic stain that has stood for some time in the laboratory is apt to be too weak to bring out this class of liposomes. These liposomes also gradually disappear under the influence of fixing reagents and post-mortem changes, but not so rapidly as the faintly refractive ones.

The term "liposomes" is used in this paper to include all droplets, visible in the fresh tissue, that are soluble in absolute alcohol and ether and are

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[1] Received May 18, 1911.

stainable by Herxheimer's scarlet-red solution.[1] The exact fatty substances that enter into the composition of the liposomes are not definitely known. It is known that triolein is often an important constituent, but no other true fats or lipoids have been certainly identified. Scarlet-red and sudan III. apparently do not distinguish true fats from certain lipoids.

The term "fatty metamorphosis" will be used in this paper to designate an increase of the microscopically visible fat[2] in a tissue, whatever may be the source of the fat. It will be the aim of this paper to bring out that in fatty metamorphosis there is an increase in the size, staining capacity, and often the number of the liposomes.

FAT CONTENT OF NORMAL MUSCLE.

It is necessary, before pathological conditions are considered, to describe the fat normally present in muscle. The literature on this topic is scanty and somewhat contradictory.

Kölliker (1857 [17]) and others have described fat in the muscle fibres of the winter frog. In 1857 Kölliker described fat droplets in the muscle fibres of man and other mammals. Ricker and Ellenbeck (1899 [21]) found no fat in the fibres of the normal muscles of adult rabbits. The earlier observers used only osmic acid as a fat stain.

Knoll (1891 [15]) described the distribution of the interstitial granules in various animals. He states that they may be dissolved by treatment with ether and alcohol, and that they may be stained more or less intensely with osmic acid. From their solubility and staining he concluded that the strongly refractive granules consist entirely of fat, and that the faintly refractive granules consist of a fatty marginal layer and a central core which may be lecithin. No explanation is offered for granules that do not stain at all with osmic acid. The papers of both Knoll (1891 [15]) and Schaffer (1893 [25]) may be taken as giving extensive accounts of the distribution of fat in muscle, since we now have convincing proof of the fatty nature of the interstitial granules.

Walbaum (1899 [29]) found fat in the muscle fibres of children in about two-thirds of the cases examined. He states that the fat content of the fibres is not influenced by the nutritive condition of the individual.

Albrecht (1902 [1] and 1903 [2]) observed that bodies similar to the interstitial granules of muscle occur in many other tissues. He believed that all these bodies consist of fat-like substances, and suggested the name "liposomes." He was apparently unfamiliar with the work of Knoll. His reasons for believing that the liposomes consist of fat-like substances seem to be as follows: (1) They are insoluble in potassium hydrate; (2) they may be converted into a substance (myelin) which is readily soluble in alcohol, ether, benzene, etc., and which may be stained a dark grey by osmic acid. He seems not to have tested the solubility of the liposomes in the fresh tissue, and he evidently regarded Altmann's fuchsinophil granules as liposomes. He did not stain the liposomes with any fat stain. Had he done so he might have seen that there is no line of demarcation between liposomes (in his sense) and ordinary fat droplets.

Babes (1908 [5]) states that fat droplets are present in the axial sarcoplasm of human cardiac fibres under apparently normal conditions. If fat be present outside of the axial sarcoplasm he would seemingly regard it as pathological.

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[1] It is to be noted that this definition of the term "liposomes" does not coincide with Albrecht's. Albrecht apparently believed that all the liposomes consist of fat-like substances—lipoids. He seemed to distinguish liposomes from true fat droplets.

[2] The word "fat" will be used as a general term to include both true fats and lipoids.

Many other observers have made incidental reference to the presence of fat droplets in muscle fibres. This point is apparently very generally recognised, but the quantity of fat present and the conditions under which it occurs are not properly appreciated. Under ordinary conditions the skeletal and cardiac muscle fibres of the cat, dog, rat, ox, chicken, frog, man, etc., contain large numbers of fat droplets—the liposomes. The liposomes are largest and brightest in the dark fibres ("trübe Fasern" of Knoll); the light fibres may not contain any. The proportion of dark to light fibres is fairly constant in any particular muscle of a given species. In any animal, therefore, the muscles that contain the most dark fibres (*e.g.*, the diaphragm and deep extensors of the thigh of the rat) will nearly always show the greatest quantity of fat. Liposomes are always present in large numbers in cardiac muscle fibres. In some animals (*e.g.*, man and the dog) the liposomes in the axial sarcoplasm of the cardiac fibres are much larger than those between the fibrillæ. Human skeletal muscle usually contains large quantities of fat. Human cardiac muscle under normal conditions may also be shown to contain large numbers of fat droplets both in the axial sarcoplasm and between the fibrillæ, if the material be obtained within a few hours after death.[1]

The confusion in regard to the fat content of muscle is due to several factors, all of which have been discussed in previous publications (Bell, 1910[6], 1911[7]).

1. *The character of the fat stain employed.*—It is well known that osmic acid does not stain all fats. At the present time oleic acid and triolein are the only simple fats that are known to be blackened by this reagent. The droplets that stain brown have not been regarded as fat by all observers. A great many droplets are not stained at all by osmic acid. It is easy to understand why the earlier observers, who relied upon osmic acid, failed to demonstrate all the fat.

It was noted above that strong simple alcoholic solutions of scarlet red or sudan III. may stain even the faintly refractive liposomes; but the stain must be at its maximum efficiency if these droplets are shown. Solutions that have stood in the laboratory for some time are apt to be too weak to bring out the fainter liposomes, since the dye precipitates to some extent. Unless special precautions are used in the preparation and control of the stain it may show only the more strongly refractive droplets.[2] Some faintly refractive liposomes are dissolved if exposed several hours to 80 per cent. alcohol; therefore a section, though it shows these droplets well-stained after fifteen or twenty minutes exposure, may show none at all if left in the stain several hours. Since the simple alcoholic solutions of scarlet-red and sudan III. are the stains generally employed, it appears that their varying action is sufficient to account for much of the difference of opinion in regard to the fat content of the tissues.

Those observers who have used Herxheimer's scarlet-red probably saw a great many of the fainter liposomes. This stain clears up the tissue so that the fainter droplets are easily seen. It does not seem to lose its capacity for staining the faint liposomes as does the simple alcoholic solution.

2. *The species of the animal.*—Some species differ markedly from others

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[1] I have examined only three fresh normal human hearts, however.

[2] Walbaum (1899[29]) states that he could see considerably more fat in the fresh tissues than he was able to stain. He used the ordinary alcoholic solution of sudan III. I now prepare the simple alcoholic solution by using a large excess of the dye (2 grms. to each 100 c.c. of 70 or 80 per cent. alcohol) and shaking vigorously for fifteen minutes. It seems better to heat the alcohol, but good results have been obtained with cold alcohol. A Herxheimer stain is always used as a control to determine when the simple alcoholic stain is bringing out all the liposomes. When maximum efficiency of staining is required the stain should be used within a few hours of its preparation.

in the staining capacity of their muscle liposomes. The liposomes in the fibres of a normally nourished cat generally stain intensely and readily. Osmic acid usually stains a majority of them. But in other animals (*e.g.*, the ox, many rats and rabbits, some dogs, summer frogs, etc.), although the liposomes may be just as numerous, they are more difficult to stain. In the animals just mentioned the liposomes are usually more weakly refractive, and are generally missed by the fat stains in common use.

3. *The character of the animal's food.*—This is a factor of the greatest importance, in some animals at least. A rat that has been fed a few days on a diet rich in fat shows a great deal more fat in its muscle fibres than does one fed on a diet poor in fat. A very striking increase in the number, size, and staining intensity of the muscle liposomes is to be seen when a summer frog is fed on fat meat or olive oil (Bell, 1911[7]). When an excess of fat is absorbed it is deposited in the muscle fibres more readily than in the connective tissue.

4. *The general nutritive condition of the animal* (*i.e.*, whether fat or emaciated).—A rat or cat when given small quantities of food containing relatively little fat, so as to cause a gradual loss of body weight, will show a gradual decrease in the number, size, and staining capacity of its muscle liposomes. The muscle liposomes of the rat are very sensitive to inanition. A twenty-four hour fast produces striking changes; and a forty-eight hour fast may remove all traces of the muscle liposomes. In the cat a high degree of inanition is necessary for the complete removal of the muscle liposomes, but marked changes are to be seen in the earlier stages. It is clear that in animals like the rat the amount of fat in the muscles visible microscopically will vary greatly from time to time in accordance with the quality and quantity of the food supply.

5. *The effect of fixatives.*—A great many of the liposomes in muscle disappear rapidly in the ordinary fixing reagents. This is particularly true of the faintly refractive liposomes. Those that are strongly refractive are usually only slightly affected, but even these may largely disappear after several weeks in formalin. Many of the liposomes in the kidney are much more sensitive to formalin than are those of muscle. The fresh tissue will usually show considerably more fat than can be seen after fixation.

6. *The effect of post-mortem changes.*—The liposomes are shown best when the tissue is examined immediately after death; but a few hours may not produce any noticeable changes. If, however, the tissues be left twenty-four hours or longer after death all the fainter liposomes will have disappeared. For this reason the human material ordinarily available shows much less fat than is present in the fresh tissue. A heart obtained a short time after death may show a picture that is commonly regarded as a fatty heart. The freshness of the material is a factor of importance in the microscopical diagnosis of fatty heart.

The amount of microscopically demonstrable fat in muscle depends, therefore, upon the species of the animal, the quantity and quality of its food, and its general nutritive condition. The amount of fat stained depends upon the stain employed and the treatment of the tissue before it is stained. Neglect of any of the six above-mentioned considerations may lead to an inaccurate estimation of the amount of microscopically visible fat.

It may be repeated here that the term "fat" is used in a general sense to include both true fats and lipoids. The exact fatty substances that enter into the composition of the liposomes are not known with any degree of certainty. The liposomes that stain black with osmic acid are probably composed essentially of triolein. Those that stain varying shades of brown with osmic acid probably contain more or less olein mixed with some other fatty substance. Those that do not stain at all with osmic acid probably consist, in part at least, of some low-melting fatty substance like butyrin. The difference in refractive power among the liposomes may be due to the proportion of true fat present, but it may also be due to the presence of different kinds of fatty bodies. It is not known to what extent lipoids enter into the formation of the muscle liposomes. Fatty acids and soaps may probably be excluded because Benda's stain always gives negative results. The muscle liposomes are isotropic and therefore do not belong to the myelin group.

FATTY METAMORPHOSIS.

As stated in the introduction, this term is used to designate an increase of microscopically visible fat. When the increase of fat is associated with the normal activity of the tissue elements it will be referred to as *physiological fatty metamorphosis*; and when the increase of fat is associated with disordered cell metabolism it will be designated *pathological fatty metamorphosis.*

Kölliker (1857 [17]) was the first to suggest that the interstitial granules of striated muscle become converted into fat droplets. He stated that the true interstitial granules do not consist of fat, but that under certain conditions (*e.g.*, the winter frog) they may be transformed into fat droplets.

Kölliker (1889 [19]) used osmic acid to stain the fat, and therefore did not recognise the fatty nature of the fainter interstitial granules. His results, however, show the increase of olein in the liposomes in physiological fatty metamorphosis. He thought that in fatty degeneration the fat droplets are formed by the disintegration of the fibrillæ.

Stuart (1865 [28]) irritated muscle by injections of silver nitrate. He states that at first albuminous droplets appear, which are later converted into osmic-staining (fat) droplets.

Knoll and Hauer (1892 [16]) described a marked increase in the refractive power and the staining capacity of the interstitial granules in the dark fibres of the pectoral muscles and in the heart muscle of pigeons after phosphorus poisoning. In the poisoned animals the granules were stained brown or black by osmic acid. This is the first clear statement that in pathological fatty metamorphosis the fat droplets are formed from the interstitial granules.

Ehrhardt (1896 [10]), in a discussion of fatty metamorphosis of the muscle fibres in trichinosis, suggests the possibility that the fat droplets may come from the interstitial granules, but inclines to the view that this is not their source.

Albrecht (1903 [2]) expressed the view that in fatty metamorphosis of the myocardium the fat droplets come from the liposomes. He states again (1907 [3]), in explanation of this process, that either the lipoids are split up into fat or that fat is passed into the liposome and substituted for the lipoids.

Elbe (1899 [11]) found fine fat droplets in the muscle fibres of rabbits that had been fattened with olive oil.

Arnold (1903 [4]) describes fat droplets in the cardiac fibres of mice after subcutaneous injections of sodium oleate. He states that the fat droplets have the same arrangement as the interstitial granules.

In a previous paper (Bell, 1911 [7]) it was shown that the fat in the muscles of the summer frog readily increases when the animal is given a diet rich in fat. A careful study of the process showed that in response to the fatty food the liposomes increase rapidly in refractive power, number, size, and staining capacity. New liposomes appear first as very small faintly refractive droplets. A similar increase of the liposomes was noted in rats that were fed heavily on fat meat or olive oil.

MATERIAL AND METHODS.

All of the experiments reported in this paper were made on rats (albino white rats and native brown rats). The method employed to produce fatty metamorphosis in the skeletal muscles was usually passive hyperæmia. This condition was obtained by tying a strong ligature around the middle of the thigh. The ligature was passed around the limb between the skin and the muscles and drawn very tightly. The skin was not included in the ligature, since, when this was done, there was usually a necrosis of the limb not attended by the accumulation of fat. Some circulation must apparently be allowed in the limb distal to the ligature if fatty metamorphosis of the muscle is to occur. In a successful operation the leg swells rapidly to about twice the normal size and shows considerable bluish discoloration. The maximum fatty condition is usually found about forty-eight hours after the application of the ligature. If left over forty-eight hours there is usually some sloughing around the ligature, with a consequent removal of the pressure and disappearance of the swelling. The increase of fat is not found unless the leg is greatly swollen for about forty-eight hours.

Frozen sections through the gastrocnemius and soleus of the normal and of the ligatured leg were made and compared. The muscle fibres of the ligatured leg show much more fat than those of the normal leg. The difference in fat content represents the amount of fatty metamorphosis produced by the ligature.

The amount of fat developed in the ligatured leg is closely dependent upon the amount of fat in the muscle at the time of the operation and the subsequent feeding of the animal. The rats experimented upon may be divided into three groups:

GROUP A.—These animals contained a comparatively small amount of fat in their muscle fibres at the time of the operation.[1] Subsequent to the operation they were full fed on starch, so that there was comparatively little loss of body weight. No fat was given in the food.

GROUP B.—These animals were full fed on fat meat and olive oil for several days before the operation. This fills the muscle fibres with fat. Subsequent to the operation they were given the same diet.

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[1] This was determined from their condition when killed. It was found from a number of experiments that in rats full fed on starch the amount of fat in the muscles does not vary appreciably for a few days at least.

GROUP C.—These rats were reduced slightly in weight before the operation. After the operation they were either given a small amount of starch or all food was withheld.

In several animals subcutaneous injections of phosphorus oil were made in order to produce fatty metamorphosis. This produced a marked increase of the liposomes of the kidney and liver, but the effect upon the muscle liposomes was not definitely determined.

A number of experiments were made in order to study the increase of the liposomes resulting from a diet rich in fat. A piece of muscle from the vastus intermedius was removed and examined at the beginning of the experiment. The animal was then heavily fed on a ration consisting mainly of fat meat or olive oil. After a few days the animal was killed. By comparing the piece of muscle removed with the same part of the corresponding muscle of the opposite leg a very accurate estimate of the increase of the fat was obtained.

Frozen sections and teased specimens were stained with Herxheimer's scarlet-red, with strong simple alcoholic solutions of sudan III., and with osmic acid. Unstained specimens were examined as controls. Fixatives were used in special instances.

Experiments.

GROUP A.—*Passive hyperæmia.* A typical experiment is here given.

February 20, 1911.—Brown rat, No. 54, weight 144·5 grms. Left leg ligatured above the knee. Fed on starch. The leg soon became greatly swollen, and showed a bluish discoloration. The swelling and discoloration persisted during the forty-eight hours the animal was allowed to live. The weight when killed was 138 grms. A small amount of connective-tissue fat was present in the abdominal cavity. The leg operated on was very much swollen. The muscles below the knee were twice as large as those of the normal leg, and were dark red in colour from the infiltrated blood. Upon teasing it was noted that the muscle fibres of the leg operated upon were much more tender than were those of the normal leg.

Frozen sections cut transversely through the gastrocnemius and soleus of each leg were stained and compared. Striking differences were apparent, particularly in the dark fibres. Plate XVIII. Figs. 1 and 2 bring out the differences. It will be noted that in the dark fibres of the normal leg (Plate XVIII. Fig. 1) the liposomes are much smaller and less intensely coloured than are those in the dark fibres of the ligatured leg (Plate XVIII. Fig. 2). The fibres of the ligatured leg are considerably larger than those of the normal leg. Some of the light fibres of the leg operated upon contain small liposomes, but there are no liposomes in the light fibres of the normal leg. The liposomes of the ligatured leg, such as are shown in Plate XVIII. Fig. 2, were stained brown by osmic acid; but no liposomes in the normal leg muscle were stained by this reagent.

Several other rats operated upon and fed as above described, gave essentially the same results.

GROUP B.—*Fat animals.—Example.* White rat, No. 59. Fed four days on olive oil and fat meat. March 11, 1911.—Weight 98 grms. Left leg ligatured. Fed as before. March 13.—Killed, weight 96 grms.

A large amount of connective-tissue fat is present. All the muscle fibres of both legs are full of liposomes. In the fibres of the leg operated upon, the liposomes are, however, larger and more brightly stained than are those of the normal leg. Plate XVIII. Figs. 3 and 4 bring out the differences exhibited by the dark fibres. The liposomes in the light fibres of the leg operated upon are somewhat brighter than are those in the corresponding fibres of the normal leg.

Group C.—*Inanition.*—When these animals were killed the loss of body weight was usually 20 to 25 per cent. The ligatured leg does not swell as much as it does in a full-fed animal. In extreme inanition no liposomes are present in either the normal or the ligatured leg. In moderate inanition the dark fibres of the leg operated upon resemble the fibre shown in Plate XVIII. Fig. 1, but no liposomes at all are to be found in the muscle fibres in other parts of the body.

It is evident that the liposomes in the fibres of the ligatured leg are always better developed than they are in the normal muscle fibres, but their size is dependent upon the amount of fat in the musculature. Plate XVIII. Figs. 1 and 2 show the differences in a rat that had a comparatively small amount of fat in its muscles. Plate XVIII. Figs. 3 and 4 show the differences in a very fat rat.

In inanition some fat seems to persist in the fibres of the leg operated upon after it has disappeared from the rest of the musculature, but in extreme inanition it is completely removed even from this situation.

The experiments given above show that fatty metamorphosis in the muscle fibres, induced by passive hyperæmia, consists in an increase in the size, staining capacity, and often the number of the fatty droplets already present. Observations made on the kidneys of rats poisoned by phosphorus have shown an increase of the liposomes entirely similar to the process just described.

These results are in harmony with the observations of those investigators who state that in fatty metamorphosis of muscle the fat droplets correspond to the interstitial granules of the normal fibre (Kölliker, 1857[17]; Albrecht, 1903[2]; Arnold, 1903[4]).

It will be recalled that there is not a complete cessation of blood flow through the ligatured leg, but the quantity of blood passing through in a given time must be greatly reduced. The consequent decrease in the supply of oxygen to the tissues is probably the immediate cause of the accumulation of fat in the fibres. The fat brought by the blood stream is not oxidised.

Ribbert (1897[20]) has shown that in focal fatty metamorphosis of the heart the fatty areas are those most distant from the arterial blood vessels. In these instances also decreased oxidation seems to be a factor of primary importance.

PHYSIOLOGICAL FATTY METAMORPHOSIS.

The occurrence of this process in the frog was referred to above. A similar growth of the liposomes may be seen when a rat is fed on a diet rich in fat.

Example.—White rat; No. 53. Kept without food one day. Weight, March 1, 1911, 83 grms. On this date a piece of muscle from the vastus intermedius of the right thigh was removed for examination. The rat was then full-fed on starch and fat meat until 9th March, on which date it was killed. The final weight was 99·5 grms. The rat was exceedingly fat when killed. Plate XVIII. Fig. 5 shows a longitudinal section of part of a dark fibre from the piece of muscle removed on 1st March. It will be noted that the liposomes are very small and faintly stained. In the light fibres no liposomes are demonstrable. Plate XVIII. Fig. 6 shows a longitudinal section of a dark fibre from the vastus intermedius of the left thigh when the rat was killed. The enormous increase in the size and brightness of the liposomes is evident. The dark fibres throughout the body resemble the fibre shown in the figure. The light fibres are full of smaller liposomes. Intermediate stages in the growth of the liposomes were seen in animals that were fed one and two days on the fatty diet. The cardiac fibres of this rat, No. 53, were filled with liposomes somewhat smaller than those shown in Plate XVIII. Fig. 6. (In all the rats thus far examined the fat content of the cardiac fibres is only a little less than that of the dark fibres of the skeletal muscles.)

The earlier stages of the experiment just described are clearly within physiological limits. Possibly the later stages are pathological. The changes produced in the liposomes by passive hyperæmia are essentially identical with those produced by a fatty diet.

THE SOURCE OF THE FAT.

This topic has attracted much attention in recent years. It has been well established that in many instances a part of the fat at least is carried to the fatty organ by the blood stream from other parts of the body (Rosenfeld). Rosenfeld (1903[22], 1904[24], 1906[23]) found also that certain organs, *e.g.*, the kidney, may show an increase of fat microscopically, although chemically there may be an actual decrease. He believes that in these instances fat already present in the tissue becomes visible microscopically. Albrecht (1907[3]) believed that in fatty metamorphosis a considerable part of the fat is formed from the lipoids in the liposomes.

My observations show clearly that some fat is already present in the tissue when pathological fatty metamorphosis begins (Plate XVIII. Figs. 1 and 3), but there is no certain evidence as to the source of the fat that produces the increase in the size of the liposomes. It is uncommon to find a tissue free from microscopical fat. In all probability the organs described by Rosenfeld as microscopically fat-free contained large numbers of liposomes.

It has been noted that in fatty metamorphosis the liposomes increase in size and refractive power, and stain more intensely. This increase of the liposomes has been produced physiologically only by feeding on a diet rich in triolein (Bell, 1911[7]). This evidence favours the view that in fatty metamorphosis the growth of the liposomes is due mainly to the accumulation of triolein.

Ciaccio (1909[8], 1910[9]) has introduced a modification of Weigert's method of staining medullated nerves by which he claims to distinguish lipoids from true fats. He believes that the true fats are unaffected by his fixative, and therefore dissolved by the fat solvents employed in embedding, but that the lipoids are rendered insoluble so that they are not removed. The paraffin sections are fixed on the slide and stained with a simple alcoholic solution of sudan III. The large fat globules of the connective tissue cells are not shown, but a great many tissues show numerous sudanophil droplets.

By making smears of triolein and oleic acid[1] on cigarette paper (Wlassak's method) I have found that both of these fats give positive results by Ciaccio's method. This method does not therefore distinguish true fats from lipoids accurately; but it is nevertheless a valuable addition to our technique.

Kasarinoff (1910 [13]) has used Ciaccio's method to distinguish true fats from lipoids in both normal and pathological tissues.

It is seldom possible to show any of the muscle liposomes of the rat by Ciaccio's technique; but by making certain modifications of his method, suggested by the previous work of Lorrain Smith, Mair, and Thorpe (1908 [26]), I have succeeded in demonstrating a large percentage of the more refractive liposomes in paraffin sections. The fresh tissues are fixed in 10 per cent. potassium bichromate,[2] twenty-four to seventy-two hours, at 37° C. They are then embedded in paraffin and stained as Ciaccio recommends. Probably the bichromate treatment must be varied in accordance with the particular fat that is to be fixed. Ciaccio's method fixes the more readily oxidisable fat. A small droplet is more readily oxidised than a large one. Probably the physical arrangement of the molecules in some droplets is more favourable to oxidation than in others. Lorrain Smith and Mair (1910 [27]) were able to fix unsaturated fats and lipoids by the bichromate treatment.

SUMMARY.

A marked fatty metamorphosis may be produced in the leg muscles of a rat by the application of a ligature around the thigh.

In the fibres of the ligatured leg the interstitial granules (liposomes) stain with greater intensity and are considerably larger than those of the normal fibres.

The size of the fat droplets in the ligatured leg depends upon the amount of fat in the body. In a very fat rat they are very large (Plate XVIII. Fig. 4); in a moderately lean rat they are much smaller (Plate XVIII. Fig. 2), and in extreme inanition they are absent.

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[1] The fats were obtained from Kahlbaum.

[2] A much better tissue fixation has recently been obtained by adding 5 cubic centimetres of acetic acid to each 100 cubic centimetres of 10 per cent. potassium bichromate. Sometimes better results are obtained by chromating at 48° C.

A rat fed on a diet rich in fat shows a rapid increase in the muscle liposomes very similar to pathological fatty metamorphosis.

In fatty metamorphosis new liposomes first appear as very small faintly staining droplets which gradually increase in size and staining capacity.

Pathological fatty metamorphosis is similar to the normal process. It consists in an increase in the size, staining capacity, and often the number of the liposomes.

A part of the fat is already present in the fibres when the pathological fatty metamorphosis begins (Plate XVIII. Figs. 1 and 3).

The increase of the size of the liposomes is probably due to the accumulation of triolein.

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DESCRIPTION OF PLATE XVIII.

All the specimens from which the drawings were made were stained with Herxheimer's scarlet red. No nuclear stain was used.

Fig. 1.—Cross-section of a dark muscle fibre from the vastus intermedius of the normal leg of a rat, No. 54, Group A. (× 800.)

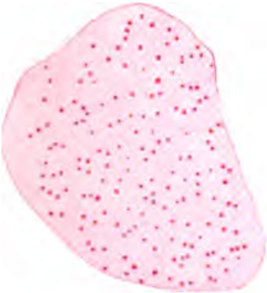
Fig. 2.—Cross-section of a dark fibre from the vastus intermedius of the ligatured leg, No. 54, Group A. (× 800.)

Fig. 3.—Cross-section of a small dark fibre from the vastus intermedius of the normal leg, No. 59, Group B. (× 800.)

Fig. 4.—Cross-section of a large dark fibre from the vastus intermedius of a ligatured leg, No. 59, Group B. (× 800.)

Fig. 5.—Longitudinal section of part of a dark fibre from the right vastus intermedius of a very lean rat, No. 53. From a piece of muscle removed at the beginning of the experiment. (× 1600.)

Fig. 6.—Longitudinal section of a dark fibre from the left vastus intermedius of rat No. 53 after eight days feeding with fat meat and starch. The dark fibres in other parts of the musculature resemble the one shown in the figures. (× 1600.)



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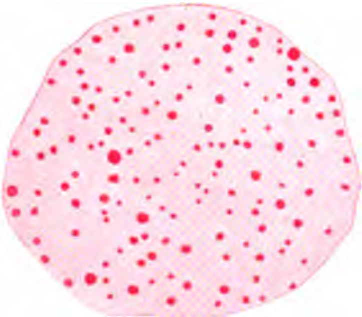

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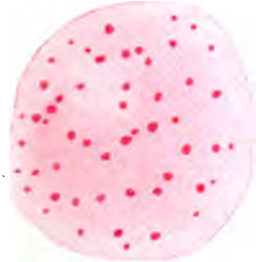

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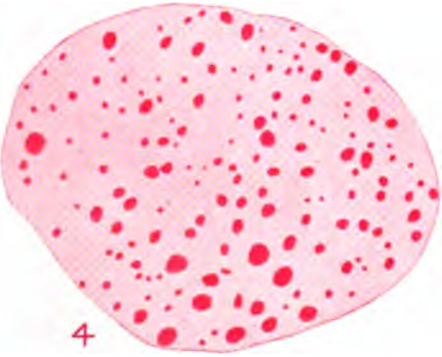

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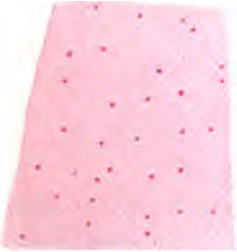

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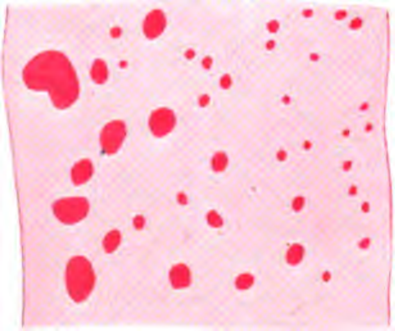

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Reprinted from The Journal of Comparative Neurology, Vol. 23, No. 2
April, 1913

NERVUS TERMINALIS IN REPTILES AND MAMMALS[1]

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TWELVE FIGURES

The table on the following pages will show in the briefest form the occurrence and relations of the nervus terminalis in various vertebrates.

In all the forms in which the nerve enters the olfactory bulb it has been shown that its fibers pierce the formatio olfactoria to pass on to their proper endings in some part of the forebrain. Where the nerve is described as entering the brain near the preoptic recess it is quite probable that the point of entrance is near the neuroporic recess as in selachians. In both cases at any rate the root has its attachment to the brain beside the lamina terminalis. From these facts it appears that the nervus terminalis in most fishes and amphibians is a ganglionated nerve whose root enters the forebrain caudal to the olfactory bulb, usually near the site of the embryonic neuropore, and whose fibers are distributed to the wall of the nasal sac. The presence of bipolar ganglion cells in the course of the nerve shows that it is in part at least a receptive nerve. Whether there are also efferent fibers of the sympathetic type (vaso-motor) or other components in the nerve can not be determined at present.

The purpose of this paper is to give a general description of the nerve as it appears in certain reptilian and mammalian embryos. I wish to acknowledge my great obligation to Dr. G. Carl Huber who has kindly given me the free use of his excellent collection of human and other embryos. The embryos studied in his laboratory are indicated below.

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[1] Neurological Studies from the Institute of Anatomy, University of Minnesota, No. 17.

AUTHOR	FORMS STUDIED	POSITION OF ROOT	GANGLION	CENTRAL RELATIONS	DISTRIBUTION
Fritsch '78	Galeus canis	dorsal surface of telencephalon			
Pinkus '94, '95	Protopterus	rec. praeopticus			nasal sac near naris
Allis '97	Amia calva	bulbus olfactorius	diffuse		nasal epithelium far forward
Locy '99	Squalus embryos	dorsally near neuropore	near root	septum	lateral part of nasal sac
Sewertzoff '02	Ceratodus embryos	near rec. praeopt.	present		nasal sac, mucous membrane, not olfactory
Locy '03, '05	selachians	dorsal or ventral	always present	septum	nasal sac
K. Fürbringer, '04	adult Ceratodus				
Bing and Burckhardt '04, '05	adult Ceratodus				
DeVries '05	human foetus 3 to 4 months	medial surface of lobus olfactorius	present		
Brookover '08, '10	Amia calva	bulbus olfactorius	composed chiefly of sympath. cells		reticulum beneath olfactory epithelium; blood vessels
Sheldon '09	Cyprinus carpio	not seen	present	decussates in com. ant.	olfactory epithelium
Herrick '09	Rana pipiens	ventral, caudal to for. bulbaris	present	nuc. medianus septi	
Döllken '09	rabbit, mouse and human embryos	at concrescentia primitiva	present	septum, gyrus fornicatus	vomero-nasal organ
Johnston '11	selachians	near rec. neurop. always			

AUTHOR	FORMS STUDIED	POSITION OF ROOT	GANGLION	CENTRAL RELATIONS	DISTRIBUTION
Brookover and Jackson '11.......	Ameiurus embryos	bulbus olfactorius	like that in Amia	com. anterior	
McKibben '11......	urodeles	bulbus olfactorius	not seen	nuc. praeopticus, hypothalmus, interpeduncular region	
Present paper.......	turtle embryos	medial surface hemisphere caudal to bulbus olfactorius	in several clumps on trunk and rami		Rostral part of medial diverticulum of nasal sac
	pig and sheep embryos	at fissura prima caudal to bulbus olfactorius	compact on root	toward com. anterior	vomero-nasal organ
	human embryos	at fissura prima caudal to bulbus olfactorius	at first compact, later diffuse		with septal strands of olfactory nerve

PIG EMBRYOS

The ganglion of the nervus terminalis (ganglion terminale) is distinguishable in the 9 mm. pig and the nerve has been seen in all the specimens examined of various stages up to 53 mm. It is most conspicuous in embryos of 15 to 25 mm. after which it seems to grow smaller. It was not seen in the later stages examined, namely, of 74 mm. and 90 mm.

In the 22 mm. embryo (fig. 1) the root of the nerve enters the brain at the ventral (rostral) end of the fissura prima (His). The root fibers are traceable for some distance caudally, i.e., toward the anterior commissure. In figure 1 only the portion of the nerve which contains ganglion cells has been modelled. The elongated ganglion terminale extends rostrally along the medial side of the root of the olfactory nerve and continues for some distance along the four primary branches into which the nerve divides. From each of the free ends of the ganglion shown in the model, strands of nerve fibers extend peripherally. In this embryo the ganglion terminale is independent of the mass of cells lying among the root bundles of the olfactory nerve known to older authors as the 'olfactory ganglion.' In other embryos the two masses of cells are frequently more closely related (fig. 5). The ganglion terminale may easily have been seen by earlier workers and considered by them to be a part of the so-called olfactory ganglion. The cells of the latter have the appearance of neurilemma cells, being slender or flattened cells taking a moderate stain in carmine. The cells of the ganglion terminale have larger, more globular nuclei, are more closely packed and take a deeper stain.

The peripheral branches of the nerve in this 22 mm. embryo pass down through the septum in several strands which end in the wall of the vomero-nasal organ and in a small area of the wall of the nasal sac immediately adjacent.

The peripheral course of the nerve is much more readily followed in sagittal sections. A model made from sagittal sections of a 15 mm. embryo is drawn in figure 3. Here the root of the nerve occupies the same position as in the previous embryo.

The ganglion is more compact and lies in the angle between the olfactory bulb and the forebrain proper. Peripherally the strands of the nerve pass through a depression in the medial wall of the olfactory sac, diverge a little from one another and converge again slightly to end in the wall of the vomero-nasal organ. One strand passes to the nasal sac rostral to the vomero-nasal organ.

In figure 2 are shown the ganglion and root as they appear in sagittal section of a 19 mm. embryo. The root fibers as they pass caudad in the brain wall are accompanied by slender elongated cells.

In the 53 mm. embryo the differentiation of gray masses in the brain has gone far enough to make it possible to determine the general relations of the root. In figure 6 A, which represents a transverse section of the rostral end of the forebrain, the mediodorsal cortex is seen extending down in the medial wall of the hemisphere and ending abruptly. The lower portion of this medial cortex is the developing hippocampus. Below it is the precommissural or paraterminal body of Elliot Smith. The boundary line between the two corresponds to the zona limitans which separates the hippocampal primordium from the precommissural body in such primitive forms as selachians. In this section the root of the nervus terminalis is seen entering the brain a little below the border of the hippocampal cortex. The 16 mm. pig (fig. 5 A) shows the nerve just above the tuberculum olfactorium. As the writer has shown elsewhere ('11), the nervus terminalis in certain typical selachians follows the zona limitans and is lost in the brain substance near the neuroporic recess. It is therefore clear that the nervus terminalis enters the brain in the pig in the same relations as in selachians. Peripherally the nerve in the 53 mm. pig bears essentially the same relations as in the younger stage described. The ganglion is more slender and the nerve gives the appearance of being reduced in size actually as well as relatively.

I have not yet been fortunate in securing a Golgi impregnation of the root of this nerve in the pig. Peripherally it takes the silver impregnation fairly well and its distribution to the vomeronasal organ has been verified by this method. A few bipolar ganglion cells connected with its fibers have been stained also. Further, a single preparation shows fibers which leave the peripheral strands of this nerve just distal to its ganglion, to join the root of the olfactory nerve and enter the olfactory bulb. This shows that the peripheral strands which have been described as nervus terminalis contain also the fibers which persist in the adult as the vomero-nasal nerve.

THE SHEEP

In embryos of the sheep of about 15 and 20 mm. the nervus terminalis is clearly present. Its ganglion is somewhat less sharply defined but in general it presents the same relations as in pig embryos.

HUMAN EMBRYOS

In an embryo in the writer's collection (H. 16) of 15.3 mm. ganglion cells appear medial to the root of the olfactory nerve in a position corresponding to that of the nervus terminalis in the pig. There are groups of ganglion cells found also in the ventral and lateral parts of the olfactory nerve. They are distinguished from neurilemma cells by their larger size, globular nuclei and deeper stain. The bundles of the nervus terminalis can not be traced in this embryo but the position of these ganglion cells suggests that the nervus terminalis may be distributed in part to the lateral wall of the nasal sac.

In an embryo of 9.5 mm. the ganglion terminale can not be distinguished as it can be in pig embryos of the same length. This embryo was received very fresh and its fixation is excellent.

The embryo XLVII of the Huber collection, 31 mm. in length, is cut in faultless sagittal sections 15μ in thickness and stained in hematoxylin and congo red. A model has been made of a sufficient portion of the right half of the brain adjacent to the median plane to show the relations of the nervus terminalis (fig. 4). The olfactory bulb is well formed and the fissura prima is begun in the medial wall of the hemisphere. From the ventromedial surface of the brain at the level of the fissura prima, and therefore behind the olfactory bulb, spring three small roots, two of them more medially and in contact with one another, the third twelve sections farther laterally. The three roots unite upon the ventro-medial surface of the olfactory nerve and there enter a distinct small ovoid or pear-shaped ganglion terminale. From the distal border of this ganglion go off strands of nerve fibers which mingle with those strands of the olfactory nerve which run down in the nasal septum. The ganglion terminale is closely connected distally with these olfactory strands, which present the appearance of a network, and the nervus terminalis is lost at this point.

On the left side in this embryo only one root was seen, the ganglion was somewhat more diffuse and more intimately connected with the olfactory strands in the septum.

Two other embryos in the Huber collection, VI and XXXIII, each between 15 and 16 mm. in length, show the nervus terminalis, but less clearly than the 31 mm. embryo. A drawing from one of them is given in figure 7. The ganglion cells do not show as clearly in these sections as in the writer's embryo of the same stage (H. 16), but they supplement the latter by showing the root in the usual position.

A fourth embryo in the Huber collection, IXL, 47 mm., shows the relations of the nervus terminalis clearly. It arises from the hemisphere just caudal to the olfactory bulb (fig. 8), passes rostrad ventro-medial to the bulb, comes in contact with the rostromedial surface of the 'olfactory ganglion' on the distal end of the bulb, and then joins the strands of the olfactory nerve so that it could not be followed farther.

It should be emphasized that in both the 31 mm. and 47 mm. embryos it is perfectly clear that the nervus terminalis joins with numerous strands of the olfactory nerve to make up the network of nerve bundles in the septum nasale. The writer was unable to demonstrate that the nervus terminalis was restricted to the immediate vicinity of the vomero-nasal organ as is the case in the pig.

EMYS LUTARIA

Two embryos of this turtle were studied in Dr. Hüber's laboratory. In embryo *F*, 10 mm. in length, the nervus terminalis arises from the rostral end of the medial wall of the hemisphere, caudal to the olfactory bulb, descends over the medial surface of the bulb and olfactory nerve and bears clumps of ganglion cells at several points of its course (fig. 9). It comes into close relation with the dorsal division of the olfactory nerve but is distinguishable from it.

The peripheral distribution of the nerve is clearer in the 20 mm. embryo (*K*), in which it becomes more closely related to the dorsal division of the olfactory nerve. As shown in figure 11, the formatio olfactoria in the bulb is divided into dorsal and ventral portions. The dorsal portion receives the dorsal division of the olfactory nerve and the appearances in the bulb alone strongly suggest comparison with the vomero-nasal nerve and the accessory bulb into which it enters in marsupials and mammals. When the dorsal division of the olfactory nerve is traced distally, however, it is found to take a peculiar course which is illustrated diagrammatically in figure 10. It remains distinct from the ventral division, passes downward in the septum over the wall of a medial diverticulum of the nasal sac, turns laterad beneath the sac and is distributed to the wall of the most lateral portion of the nasal sac, situated beneath the orbit. This course and distribution seems to preclude the possibility of comparing this dorsal division of the nerve with the vomero-nasal nerve of mammals. The medial diverticulum of the nasal sac in this embryo seems to be the beginning of the vomero-nasal organ, and this is innervated by the ventral division of the olfactory nerve and in part by the nervus terminalis (fig. 10).

The nervus terminalis appears in sagittal sections rather far dorsally in the medial wall of the hemisphere (fig. 12 A). It passes forward over the medial wall of the bulb, converging with its fellow until they touch where the tips of the olfactory bulbs approach closest to the median plane (fig. 12 C). The nerve then passes alongside the dorsal division of the olfactory nerve and becomes somewhat mingled with it. At intervals groups of ganglion cells appear in the dorsal division, which the writer attributes to the nervus terminalis (fig. 12 D, E). As the olfactory nerve approaches the nasal sac, its ventral division spreads out to innervate the medial diverticulum as well as the dorso-lateral wall (fig. 10, 12 D). The dorsal division breaks up into several strands which pass downward over the medial surface of this diverticulum to run beneath the nasal sac and reach its extreme lateral portion as above described. Meantime several small strands leave the dorsal division and bend rostrad over a partly separate rostral portion of the medial diverticulum, to which they are distributed. These small strands bear clumps of ganglion cells (fig. 12 E) which mark them as strands of the nervus terminalis.

Taking both the 10 and 20 mm. embryos into account, it may be said that the ganglion terminale in Emys is scattered in clumps of variable size along the nerve from its root to the terminal branches near the nasal sac.

The foregoing description shows that in the embryos of certain reptiles and mammals the nervus terminalis enters the brain at a point somewhat removed from the median plane but otherwise holding the same relation to the primordium hippocampi, precommissural body and neuroporic recess which the root holds in selachians. Its fibers arise from bipolar ganglion cells which are collected into a compact ganglion terminale (pig) or are gathered into several clumps in the course of the nerve and its branches (turtle). In the pig the nervus terminalis is clearly distributed to the vomero-nasal organ and in the turtle to a medial diverticulum of the nasal sac which presumably corresponds to the vomero-nasal organ or a part of it. In man the fibers mingle with the olfactory strands of the nasal septum.

Two authors have dealt with the nervus terminalis in mammals, de Vries ('05) and Döllken ('09). De Vries' paper is not accessible to me, but from the references to it by other authors it appears that he recognized a vomero-nasal nerve and vomero-nasal ganglion whose root entered the medial surface of the rhinencephalon caudal to the bulbus olfactorius. He saw also ganglion cells scattered in the course of the nerve. He interpreted the entire structure as the equivalent of the nervus terminalis of fishes.

Döllken's paper has come into my hands since the present manuscript was finished and the figures drawn. Döllken's description of the nervus terminalis and ganglion terminale in mouse, rabbit, and human embryos is in essential agreement with the above account for the pig, sheep and man. The central connections of the root may be passed over briefly. Only Döllken's root *c* ending in the septum corresponds to the root seen by me. The continuity of nervus terminalis roots with fibers leading to the gyrus fornicatus and hippocampus seems to the writer to require confirmation. It is not clearly demonstrated in Döllken's figures.

Döllken apparently regards the nervus terminalis as the special nerve of the vomero-nasal organ. He describes a part of its fibers at least as arising from cells in the vomero-nasal epithelium. He uses the name ganglion terminale as synonymous with 'ganglion vomero-nasale' of de Vries and 'Nebenbulbus' of v. Gudden, Kölliker and others. "Am oralen Ende der Hemisphäre liegt medial das Ganglion terminale (Ganglion nasale, Ganglion vomero-nasale, Nebenbulbus)." From this it would appear that he considers the ganglion terminale as the primary center for the vomero-nasal nerve and he has been so understood by McCotter ('12, pp. 302, 316). This is, however, wholly inconsistent with Döllken's clear description of *roots* from the ganglion terminale to the paraterminal body and other regions of the hemisphere, and with his comparison of the cells of the ganglion terminale with those of spinal and cerebral ganglia (p. 23). On the other hand, it appears very probable to the writer that Döllken has failed to recognize the clear distinction which exists between the ganglion terminale and the cells lying among the olfactory nerve fibers ('olfactory ganglion') which later produce neurilemma cells. Comparison of his figures 1 and 2 with the conditions in the pig (figs. 1 and 3 of this paper) suggests that Döllken has in view in these stages of the mouse chiefly or solely olfactory fibers entering the formatio bulbaris and that the ganglion terminale of these figures is only the 'olfactory ganglion' of older authors. The apparent reduction in the number of ganglion cells in the course of the nerve in later mouse embryos (p. 17) would be accounted for on the supposition that the cells seen in early stages had developed into neurilemma cells. In Döllken's figures 4 and 22 the nervus terminalis is distinct from the olfactory nerve and the root *c* of these figures corresponds to the only root seen by the writer. The fibers of Döllken's root *a* (fig. 3) appear in my preparations to belong to the olfactory nerve.

Döllken clearly confirms the work of earlier authors both as to fibers arising from the cells of the vomero-nasal epithelium and as to free nerve endings in this organ. He says:

> Im Ruysch'schen Gang (Jakobson'schen Organ) und zwar nie an der Schleimhautoberfläche finden sich spindelförmige Zellen mit einer Fibrillenkontur, die einen Fortsatz in die Nervenstrecke des Nervus terminalis senden (Taf. IV, Fig. 16). Ausserdem laufen glatte Fasern vom Nerven bis zur Oberfläche der Schleimhaut, ohne in Verbindung mit einer Zelle des Sinnesorgans zu treten. Diese Fasern sind auch von v. Lenhossék u. A. gesehen und beschrieben worden. Sie kommen in der ganzen Riechschleimhaut vor. Manche Autoren haben die Vermutung geäussert, es könne sich um Trigeminusfasern handeln. Für die entsprechenden frühen Fasern des Ruysch'schen Ganges glaube ich diese Annahme ausschliessen zu können. Sie stammen von Ganglienzellen des Terminalnerven.

From this it is clear that two kinds of fibers are present in the nervus vomero-nasalis of de Vries and nervus terminalis of Döllken, some arising from true olfactory sense cells in the vomeronasal organ as described by v. Brunn ('92), v. Lenhossek ('92) and Read ('08), and others arising from the cells of the ganglion terminale.

The vomero-nasal nerve of mammals is not the homologue of the nervus terminalis of fishes. The two nerves exist side by side in mammalian and reptilian embryos. The vomero-nasal nerve arises from cells in the nasal mucosa indistinguishable from typical olfactory sense cells, while the nervus terminalis arises from bipolar ganglion cells situated on the course of the nerve and resembling cerebro-spinal ganglion cells. The vomero-nasal nerve enters the bulbus olfactorius where it has a special part of the formatio olfactoria as its center (the so-called accessory bulb or the formatio vomero-nasalis). The nervus terminalis enters the hemisphere caudal to the bulb in entirely different relations. The nervus terminalis in mammalian embryos is clearly a ganglionated nerve connected with the neuroporic region of the forebrain and supplying free nerve endings to the vomero-nasal organ and probably to a variable part of the nasal sac. The olfactory fibers arising in the vomero-nasal organ run in the same bundles with the nervus terminalis, but enter the bulbus olfactorius.

The existence of this nerve in adult fishes and amphibians and in embryonic reptiles and mammals warrants a careful search for it in adult reptiles and mammals. Already McCotter ('12) has found strands connected with the vomero-nasal nerve in the adult dog which enter the brain in the proper position for the nervus terminalis. Further studies may show this nerve present in adult turtles and snakes and in various mammals. It is probable that this region of the adult human brain has never been studied in any way that is favorable to the discovery of this nerve if it should be present.

Discussion of either the morphological or physiological significance of the nervus terminalis would not be profitable at this time. It is clear that there exists in the most anterior vertebrate segment a receptive nerve in addition to the olfactory. The olfactory and the nervus terminalis may be regarded as two components of a segmental nerve, analogous to the gustatory and general cutaneous components which exist together in the VII or the IX cranial nerves in some fishes. That the olfactory nerve and nervus terminalis have come to have separate roots and very widely differentiated centers is no bar to this view. For the two components in the VII or IX, or in any nerve, have differentiated centers and in many cases the fibers of one component in a cranial nerve become segregated at their entrance into the brain so as to form one or more pure rootlets. It is possible that the nervus terminalis contains one or more other components still. Brookover's work on Amia seems to show that this nerve has a relation with the head sympathetic and that the larger part of the cells in its course are sympathetic cells concerned probably in vaso-motor functions. About forty fibers constitute the forebrain root of the ganglion terminale. These Brookover would regard as preganglionic sympathetic fibers. This interpretation would not be tenable if these fibers are the axones of cells in the ganglion as he implies in his conclusions (p. 114). If they are axones of peripheral ganglion cells, they are best regarded as receptive fibers, as the writer has considered them in other fishes. That some of the cells derived from the olfactory placode along with the ganglion terminale should develop into sympathetic ganglion cells is no more than might be expected in view of the origin of sympathetic ganglion cells from the spinal ganglia. Whether any sympathetic roots remain connected with the telencephalon as a component of the nervus terminalis is still uncertain.

The continuation of some of the fibers of this nerve in urodeles to the hypothalamus and probably to the interpeduncular region of the mesencephalon as described by McKibben is very remarkable. Taken together with the apparent absence of a ganglion terminale this suggests the hypothesis that these may be efferent (sympathetic) fibers such as Brookover supposed to be present in Amia. Upon this point the writer would offer the observation that in a 35 mm. larva of Amblystoma punctatum collections of ganglion cells are found on two of the three branches of the olfactory nerve which supply the vomero-nasal organ. In view of the discussion over the vomero-nasal organ of amphibians it should be said that the development of the organ has been traced in Amblystoma. It arises as a medial diverticulum from the ventral part of the thick olfactory epithelium of the nasal sac a short time before the choanae are open into the mouth. Later a rotation of the nasal sac and the vomero-nasal organ takes place, such that this organ comes to hold the relations of a lateral diverticulum in the 35 mm. larva. The diverticulum very early gives rise to branched tubular glands which extend medially and lie ventro-medial to the nasal sac. The nerve supply here described is further evidence that this diverticulum is the homologue of the vomero-nasal organ of reptiles and mammals, as held by Burckhardt, Seydel, Hinsberg and others. (For the literature, see Peter '02). The small ganglia mentioned above lie near the vomero-nasal organ and correspond to the ganglia seen near the ends of the branches of the nervus terminalis in turtle embryos. As described by McKibben the nervus terminalis is imbedded in the olfactory nerve and its peripheral course was not seen. The ganglia on vomero-nasal branches of the olfactory nerve in Amblystoma serve to identify the nervus terminalis peripherally, and show both that the nerve is ganglionated in urodeles and that it has the same distribution as in reptiles and mammals.

The evidence at present in hand seems to establish beyond doubt the presence in all vertebrates of a receptive component in the nervus terminalis supplying ectodermal territory. This component is derived either from the terminal part of the neural crest (Johnston '09 b, Belogolowy '12) or from the olfactory placode (Brookover '10). The nerve is distributed to the nasal mucosa or to a specialized part of it, the vomero-nasal organ. What is needed now is definite knowledge of the central connections and experimental evidence as to its function.

One further suggestion, although very vague in its present form, may be hazarded here. The close association of the nervus terminalis with the vomero-nasal organ in amphibians, reptiles and mammals suggests that the influence of this nerve in the fish-like ancestors of these forms may have been an important factor in the differentiation of the vomero-nasal organ. Also it may be supposed that the distribution of the nervus terminalis in fishes would give some indication as to the portion of the nasal sac from which the vomero-nasal organ has been derived.

SUMMARY

In embryos of the turtle, pig, sheep and man there is found a true nervus terminalis consisting of a ganglionated nerve whose root enters the telencephalon caudal to the bulbus olfactorius.

This nerve exists in addition to the nervus vomero-nasalis in mammals, and the nervus terminalis is distributed chiefly to the vomero-nasal organ. The olfactory fibers arising from the vomero-nasal organ separate from the nervus terminalis to enter the olfactory bulb.

The nerve root enters the brain in the line of separation—zona limitans—between the hippocampus above and the precommissural body and tuberculum olfactorium below.

In Amblystoma the nervus terminalis is ganglionated and supplies the vomero-nasal organ as in reptiles and mammals.

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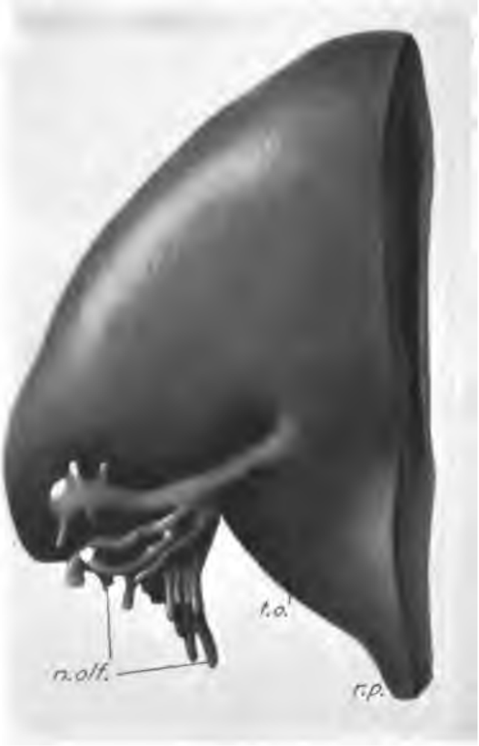
ABBREVIATIONS

b.o., bulbus olfactorius
c., choana
c.a., commissura anterior
f.i., foramen interventriculare
f.o.d., formatio olfactoria, dorsal portion
f.o.v., formatio olfactoria, ventral portion
g.t., ganglion terminale
hem., hemisphere
m., margin of lamina supraneuroporica
med.div., medial diverticulum of nasal sac
n.o.a., nucleus olfactorius anterior
n.olf., nervus olfactorius
n.olf.d., nervus olfactorius, dorsal root
n.olf.v., nervus olfactorius, ventral root
n., narial opening
n.sac, nasal sac
n.t., nervus terminalis
olf.ep., olfactory epithelium
pal., palate
p.h., primordium hippocampi
r.n., recessus neuroporicus
r.p., recessus praeopticus
s., corpus striatum
t., tectum mesencephali
t.c., tela chorioidea
t.o., tuberculum olfactorium
v.l., lateral ventricle
v-n.o., vomero-nasal organ
v.tr., velum transversum

Fig. 1 Pig, 22 mm. Model of rostral part of right hemisphere, seen from the medial surface. The bulbus olfactorius is not clearly delimited by grooves but the root of the olfactory nerve shows its position. The ganglion terminale accompanies the root and four branches of the nervus terminalis which runs obliquely across the roots of the olfactory nerve. The point *r.p.* in the model is just above and rostral to the preoptic recess.

Fig. 2 Pig, 19 mm. Sagittal section through the rostral part of the left hemisphere. The spindle-shaped ganglion terminale is closely related to a broad collection of lightly staining cells which follows the contour of the bulbus olfactorius. These latter cells belong to the neurilemma cells of the olfactory nerve. Note that the root accompanied by flattened cells is directed toward the neuroporic recess.



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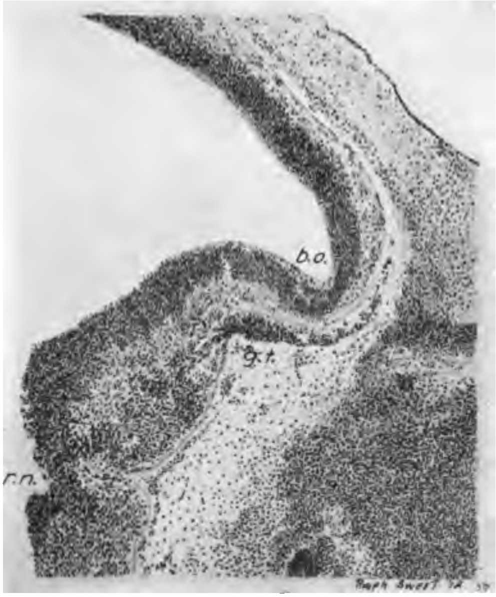

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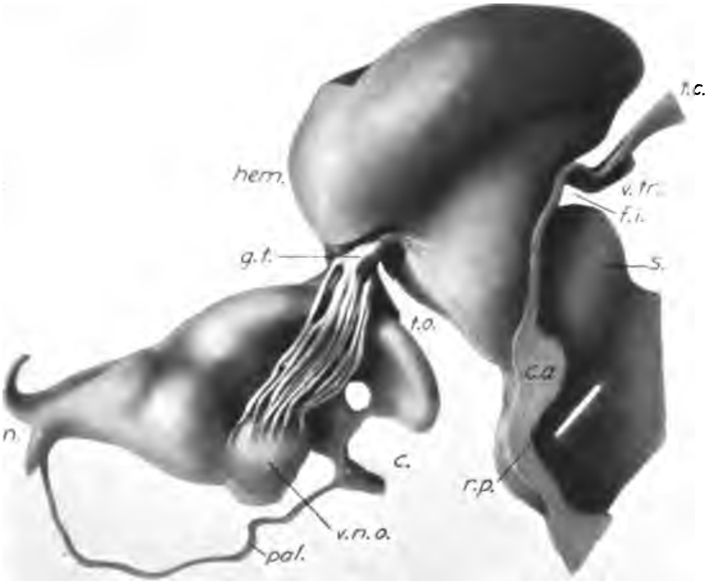
Fig. 3 Pig, 15 mm. Model of part of the right hemisphere and nasal sac, seen from the medial surface. The model includes the optic stalk, whose slit-shaped cavity is seen, and the medial wall and frontal pole of the hemisphere. The groove in the hemisphere is the fissura prima of His. The root of the nervus terminalis is directed backward in the paraterminal region. The olfactory nerve forms a dark background for the ganglion terminale in the drawing. It is at this point that olfactory fibers coming from the vomero-nasal organ separate from the nervus terminalis fibers and enter the olfactory bulb in the olfactory nerve roots. The two kinds of fibers run together in the bundles which go to the vomero-nasal organ.

Fig. 4 Human embryo of 31 mm. (Huber collection XLVII). Model of rostral portion of right hemisphere showing the relations of the nervus terminalis. The ganglion terminale is distinct from the root of the olfactory nerve but distally the nervus terminalis fibers mingle with olfactory nerve fibers.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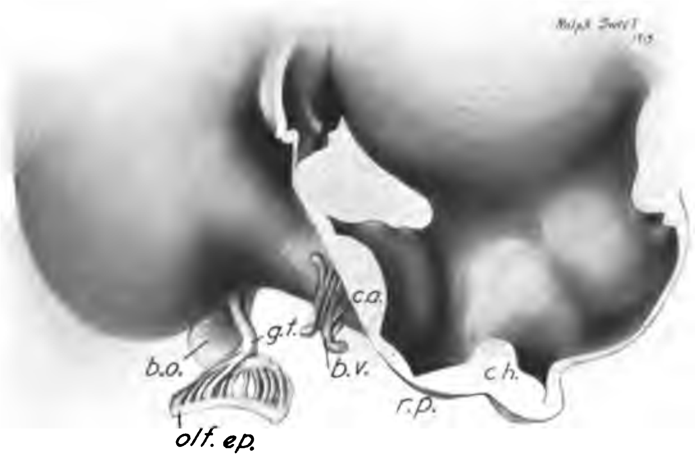
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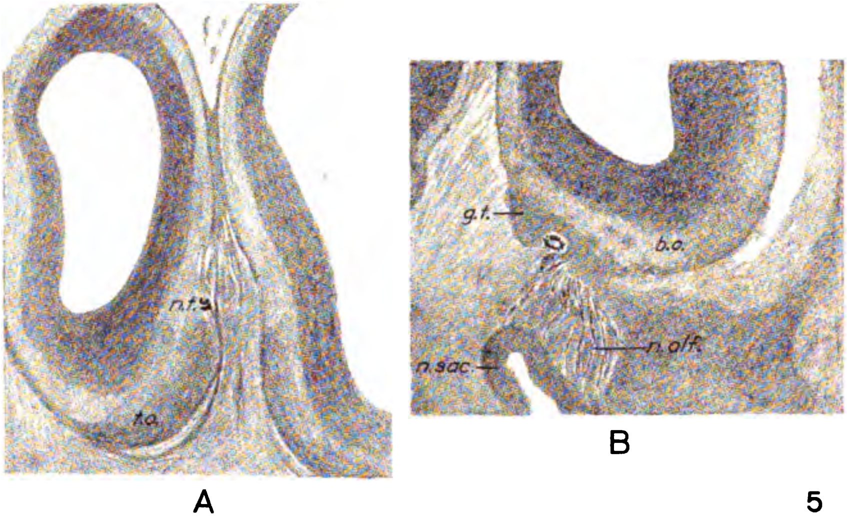

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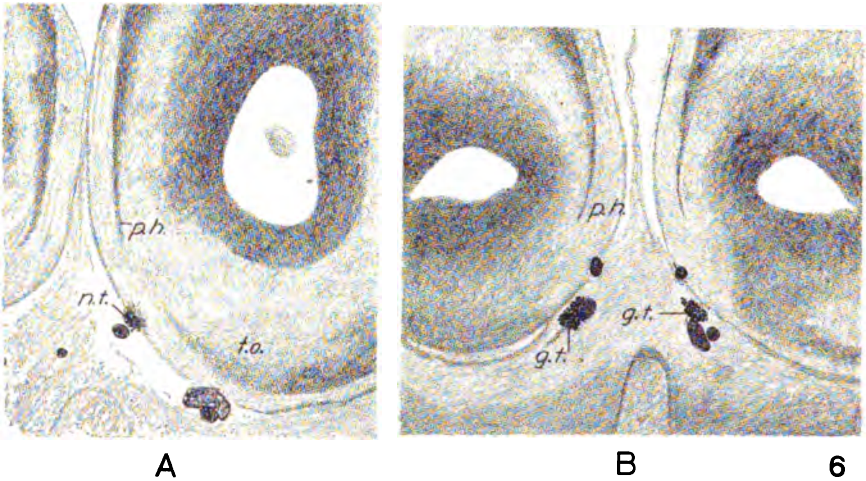

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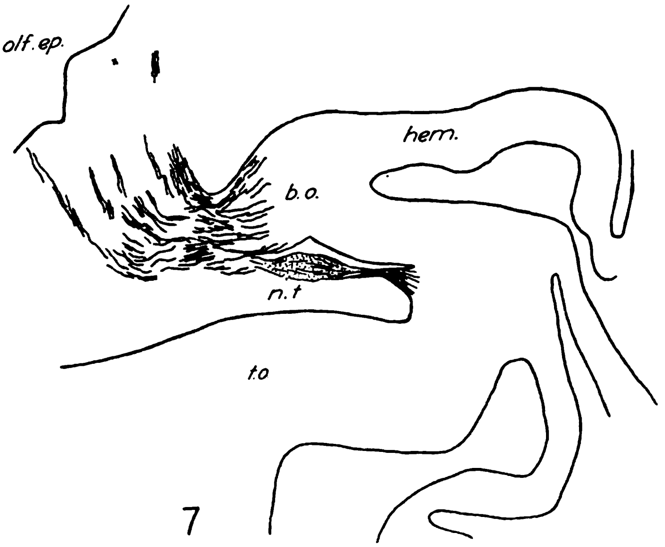
Fig. 5 Pig, 16 mm. Two transverse sections of the rostral part of the hemispheres. In *A* the root of the nervus terminalis is seen as a small collection of cells lying within the brain beneath the fissura prima and above the tuberculum olfactorium. In *B* the ganglion terminale is shown where it comes in contact with the root of the olfactory nerve. The ganglion cells are separated from the neurilemma cells of the olfactory nerve by a small blood vessel. The ganglion terminale is unusually large in this specimen.

Fig. 6 Pig, 53 mm. Two transverse sections through the hemispheres showing the root and ganglion of the nervus terminalis. Note that the root enters the brain between the primordium hippocampi and the tuberculum olfactorium.



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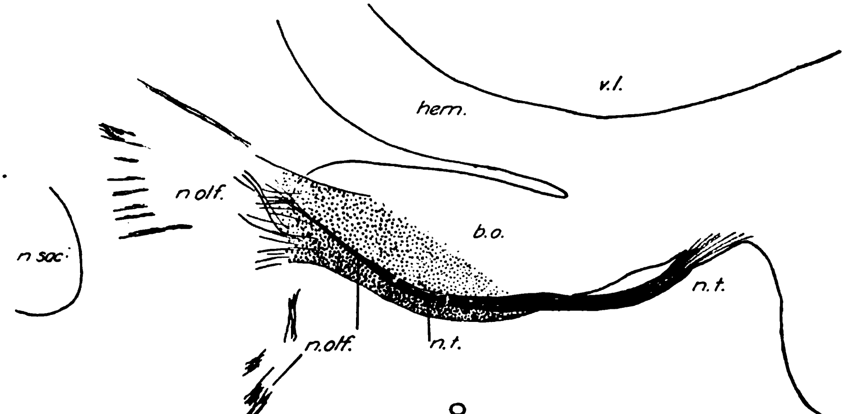

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Fig. 7 Human embryo of about 15 mm. (Huber collection xxxiii). Sagittal section through the root and ganglion of the nervus terminalis. Peripherally the nerve is lost in the olfactory bundles, but its ganglion is distinct and the root enters the brain in the characteristic position.

Fig. 8 Human embryo of 47 mm. (Huber collection ixl). A projection upon one plane of the course of the nervus terminalis from its root to the point where it mingles with the olfactory bundles. The area coarsely stippled indicates the position occupied by the mass of neurilemma cells among the root bundles of the olfactory nerve. The nervus terminalis runs over the medial surface of this mass and joins olfactory bundles running in the same general direction. Only a few olfactory fibers are sketched.

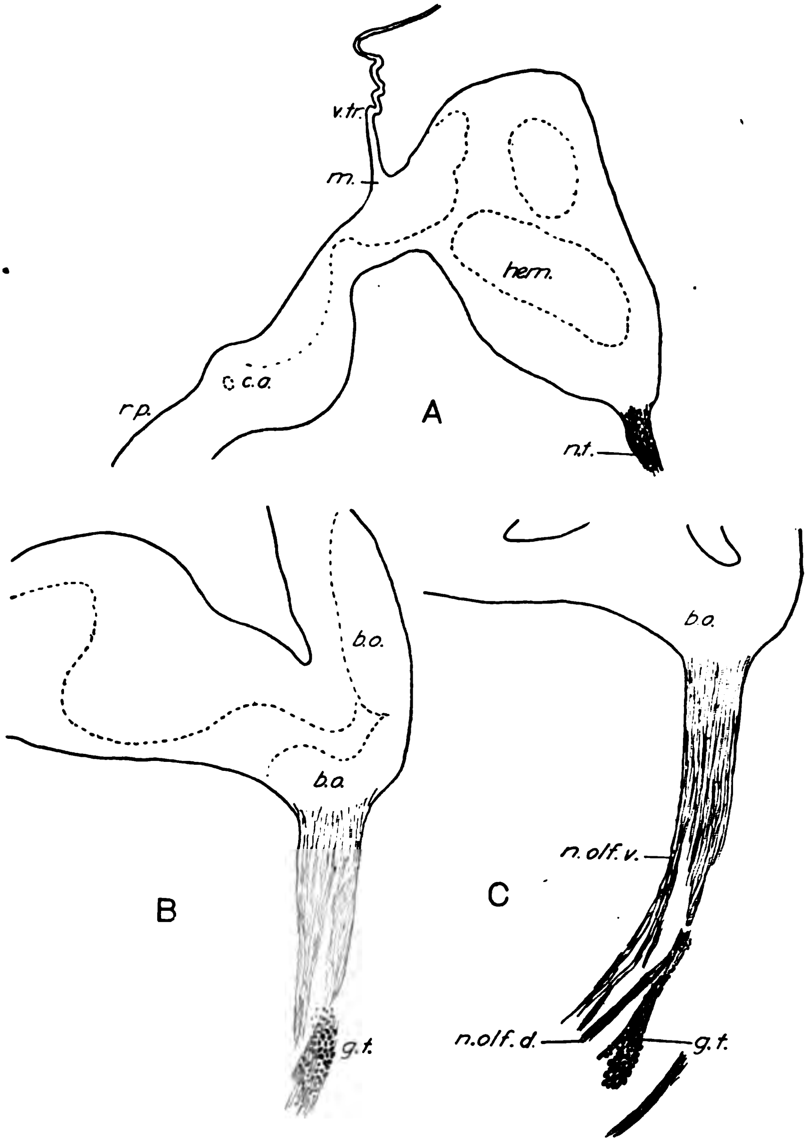
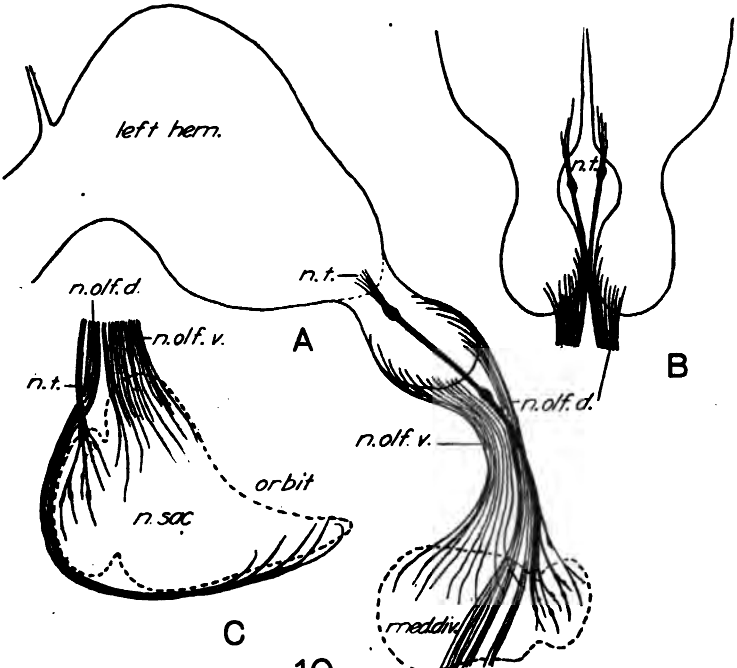


Fig. 9 Emys lutaria, 10 mm., three parasagittal sections. In *A* the nervus terminalis enters the rostral end of the hemisphere on its medial surface. It contains some ganglion cells. In *B* the olfactory bulb and ventral part of olfactory nerve are shown. The section passes through a ganglion midway of the length of the nervus terminalis. In *C* a peripheral ganglion is cut at the point where the nervus terminalis separates from the dorsal division of the olfactory nerve to enter the wall of the medial diverticulum (vomero-nasal organ).



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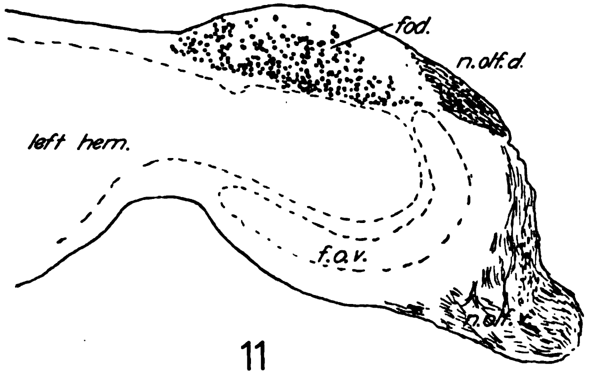

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Fig. 10 Emys lutaria, 20 mm. Three diagrams to illustrate the course of the olfactory nerve and nervus terminalis. In *A* the nerves are projected on a parasagittal plane, seen from the medial direction, *B*, a projection on the horizontal plane, ventral part of olfactory nerve omitted, *C*, a diagrammatic transverse section of the nasal sac. That part of the nasal sac to which the nervus terminalis is distributed is believed to be the vomero-nasal organ.

Fig. 11 Emys lutaria, 20 mm. Parasagittal section showing the dorsal root of the olfactory nerve and the division of the formatio olfactoria into dorsal and ventral portions.

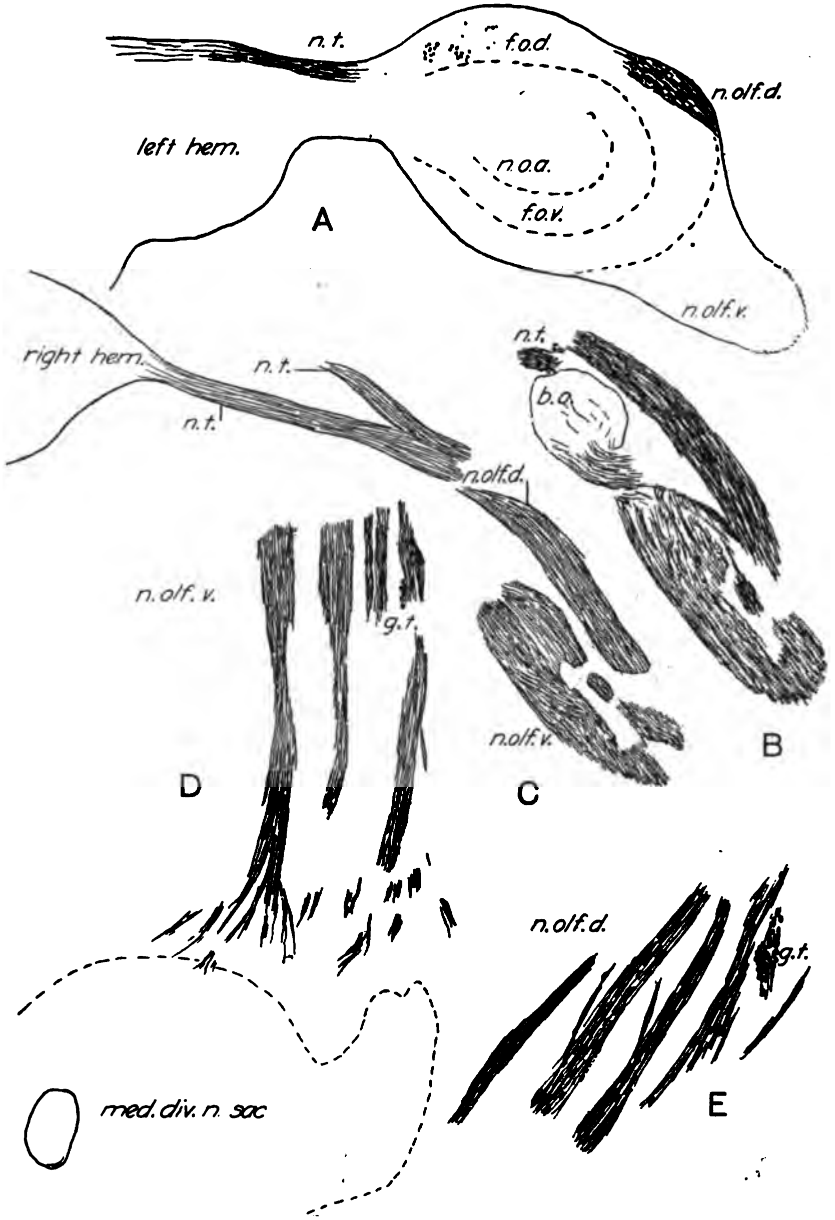

Fig. 12 Five sections from the same series as figure 11. In *A* is shown the root of the nervus terminalis entering the hemisphere behind the olfactory bulb. In *B* the nerve is almost in contact with the tip of the olfactory bulb and runs close to the dorsal olfactory root. In *C* the nervus terminalis touches its fellow (compare figure 10 *B*). In *D* the section cuts an isolated ganglion on the course of the nervus terminalis. The ventral olfactory is spreading out to its endings. In *E* the dorsal olfactory courses ventrad over the surface of the medial diverticulum. One of the small bundles of the nervus terminalis is seen with ganglion cells.

Reprinted from The Anatomical Record, Vol. 6, No. 11,
November, 1912

ON THE TELEOSTEAN FOREBRAIN

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THREE FIGURES

In the Journal of Comparative Neurology for June, 1912, appears a paper on the olfactory centers of teleosts by Dr. Sheldon, from Professor Herrick's laboratory in the University of Chicago. This paper was submitted for publication just before that by the writer ('11 b) which appeared in the same Journal for December, 1911. Dr. Sheldon and the writer carefully examined each other's manuscripts in July, 1911. Dr. Sheldon's paper presents the results of very careful study of a large number of specimens of the carp brain prepared by the most approved methods. It is the object of this note to point out the agreement as to facts in the two papers mentioned, and to comment briefly on the differences of interpretation of those facts.

The following table will indicate at once the corresponding structures as described in the two papers:

Johnston	*Sheldon*
Bulbus olfactorius	Formatio bulbaris and nucleus olfactorius anterior
Nucleus olfactorius medialis	Corpus praecommissurale Nucleus medianus Pars commissuralis Pars supra-commissuralis Pars intermedia
Nucleus olfactorius lateralis	[Lateral part of the nucleus medianus of the corpus praecommissurale.]
Primordium hippocampi	Nucleus olfactorius dorsalis Nucleus olfactorius lateralis Lobus pyriformis Nucleus taeniae Palaeostriatum

Johnston	*Sheldon*
Lobus pyriformis	[Lateral part of nucleus medianus; plus the nucleus commissuralis lateralis?]
Somatic area	Nucleus entopeduncularis and diffuse cells about it and perhaps the nucleus commissuralis lateralis.
Nucleus praeopticus	Nucleus praeopticus parvocellularis. The nucleus magnocellularis seems to be a special development in teleosts.

It is well known that there are in the adult teleost brain a much larger number of discrete nuclei and fiber bundles than are seen in the brains of other fishes. This gives the appearance of a high degree of specialization. Dr. Sheldon's description of the adult brain of a single teleost shows these numerous discrete gray masses and fiber bundles, while the writer's description, based upon several ganoids and young teleosts, seems at first sight to differ greatly because of the small number of centers and tracts described. That this is the chief difference between the descriptions given in the two papers will appear from an examination of the several structures seriatim.

In the olfactory bulb most authors recognize a nucleus olfactorius anterior. While I have described the same gray matter under the name of deep cells (stellate cells, granules, etc.), I have insisted that these belong to the formatio bulbaris because their dendrites come into relation with olfactory fibers in the glomeruli and their neurites proceed as fibers of the olfactory tract to the secondary olfactory centers. Sheldon fully and clearly confirms this relation to the olfactory fibers. He describes also a tractus olfactorius ascendens arising in the precommissural body and ending in relation with these deep cells. Sheldon speaks of the nucleus olfactorius anterior as secondary olfactory tissue but his description does not show that any olfactory tract fibers end in it.

The medial olfactory nucleus or precommissural body presents practically the same condition in the forms studied by Dr. Sheldon and myself. The 'pars intermedia' of Sheldon's description which connects the precommissural body with the central gray of the thalamus, is less conspicuous in ganoids but is present. It should be noticed, however, that Dr. Sheldon seems to include in the nucleus medianus of the precommissural body not only the anterior part of the medial olfactory nucleus but also the lateral olfactory nucleus of my description. This is a serious difference of interpretation, the significance of which will be referred to later.

In the writer's description the large dorsal part of the lateral lobe of the forebrain is given the name primordium hippocampi. The grounds for this are that this area is characterized by peculiar pyramidal cells with spiny dendrites, receives olfactory fibers of the second and perhaps of the third order, and receives ascending fibers from the hypothalamus which are believed to be gustatory in function. The cells of the peculiar type described are present throughout all parts of the area in question and the ascending fibers from the hypothalamus spread uniformly throughout the whole area in ganoids and young teleosts. The primordium is bounded medially by the sulcus limitans hippocampi in the ventricular surface and laterally by the external sulcus and the lateral olfactory tract. In the substance of the lateral lobe there is always visible a more or less conspicuous and extensive cell-free zona limitans which separates this area from the medial and lateral olfactory nuclei.

In the adult carp Sheldon describes in the corresponding area several more or less distinct gray masses and details the fiber tracts connected with each one. The boundaries of the area as a whole agree with those given by the writer. The ventricular sulcus is given the name fissura limitans telencephali. In this area Sheldon recognizes a nucleus olfactorius dorsalis occupying the dorso-medial angle of the everted lateral lobe and adjoining the precommissural body; a nucleus olfactorius lateralis which includes the greater part of the entire structure; a lobus pyriformis and a nucleus taeniae adjacent to the taenia fornicis in the caudal part of the lateral lobe; and a palaeostriatum deeply imbedded in the lateral lobe, beneath the nucleus olfactorius lateralis. Dr. Sheldon's figures 32, 33, 45, 48, 49, 50, 51, 52, 53, 58, 59, 60, show the cells with the peculiar spiny or thorny dendrites in all the nuclei mentioned except in the nucleus olfactorius dorsalis. In the text (p. 193) it is stated that many of the cells of this nucleus "resemble the dorsal cells of the nucleus olfactorius lateralis (figs. 48, 49, 56)." So far as the types of cells are concerned, all the nuclei mentioned might well be regarded as parts of one large center. This is a very welcome confirmation of the writer's description of the primordium hippocampi in fishes, because the existence of these cells of a peculiar type in the primordium hippocampi of various fishes and amphibians has been used as one of the chief means of identifying the primordium hippocampi in cyclostomes ('02 a, '06, and '12 b[1]).

With regard to the various fiber tracts connected with these several nuclei, there is noticeable a close similarity between all parts of the area which I have called primordium hippocampi. All parts receive olfactory tract fibers, those from the medial tract entering the medial border those from the lateral tract entering the lateral border. There are decussating fibers also entering the lateral border. Sheldon has described further tertiary olfactory fibers from the precommissural body entering the nucleus olfactorius dorsalis and the palaeostriatum. Although such tertiary fibers are probably present in cyclostomes ('12 b), they are proportionately few in selachians and have not been certainly demonstrated heretofore in teleosts. It can not be argued that the term primordium hippocampi must be limited to that part of the forebrain into which these tertiary olfactory fibers enter.

All the nuclei mentioned, except the dorsal olfactory nucleus, receive ascending fibers from the hypothalamus by way of the tractus olfacto-hypothalamicus lateralis of Kappers or by way of the tractus strio-thalamicus. All of these ascending fibers evidently correspond to the tractus pallii of the writer in ganoids and young teleosts. In several of the forms studied by the writer (Polyodon, Ameiurus) there are large bundles of the tractus pallii rising into the caudal part of the primordium hippocampi. These would correspond to the ascending fibers in the tractus olfacto-hypothalamicus lateralis, although they are less completely segregated than in the carp. The ascending fibers in the tractus strio-thalamicus in the carp have a distribution similar to that of the larger part of the tractus pallii in the forms described by the writer. It is to be noted that ascending fibers from the hypothalamus running in the medial forebrain bundle go up to the medial border of the primordium hippocampi in the sturgeon, partly direct and partly after crossing in the anterior commissure. Sheldon traces similar fibers only as far as the precommissural body.

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[1] Johnston, The telencephalon in Cyclostomes, Jour. Comp. Neur., vol. 22, August, 1912.

Descending fibers are described by Sheldon from all these nuclei to the hypothalamus by way of the medial or lateral hypothalamic tract or the tractus strio-thalamicus. This confirms the early description by Van Gehuchten and is in agreement with the writer's findings in the small number of brains of Amia in which such fibers were impregnated. The unequivocal description by Sheldon removes all doubt as to the descending pathway from the primordium hippocampi in teleosts. This is also in agreement with the conclusion reached by several authors that the tractus pallii in selachians is composed of both ascending and descending fibers.

Finally, a tract called the tractus taeniae arises from the nucleus taeniae and runs to the nucleus habenulae. This is said to be the morphological equivalent of the tractus cortico-habenularis lateralis of Herrick in the frog. It is possible that this part is accompanied by a few commissural fibers constituting a posterior pallial commissure. This tractus taeniae of Sheldon together with his somewhat hypothetical posterior pallial commissure corresponds to what the writer has termed the posterior pallial commissure. Although a true commissure has not been certainly demonstrated by either of us, the presence of such a commissure in cyclostomes (Johnston '12 b), selachians, amphibians and reptiles, renders its existence in ganoids and teleosts very probable.

Both the medial and lateral borders of the general area are connected by commissures which Sheldon calls the anterior and posterior hippocampal commissures. In Amia there is a single large commissure distributed to the whole area.

There is thus seen to be a close agreement as to facts in the description of the general area under consideration. Dr. Sheldon, however, interprets this general area as including the lateral olfactory nucleus, the pyriform lobe, the nucleus taeniae and a palaeostriatum, while only the nucleus olfactorius dorsalis is regarded as the primordium hippocampi. Since the whole area has so nearly identical structure and connections throughout its parts, this interpretation seems to the writer untenable.

First, the commissures, the habenular and the hypothalamic connections all point more clearly to the lateral part of this area as the homologue of the primordium hippocampi in cyclostomes, selachians and amphibians. It is only in the entrance of tertiary olfactory fibers that Sheldon's dorsal olfactory nucleus bears a special resemblance to hippocampus. This is the least important characteristic of the hippocampal primordium in lower forms and in the carp it is shared with the palaeostriatum.

Second, most writers on the teleost brain in identifying the lateral olfactory nucleus have ignored the important fact of eversion. They regard the structures which are laterally placed as morphologically lateral. The position of the line of attachment of the tela chorioidea, the form of the lateral lobes, the position of the zona limitans, all show that the so-called lateral olfactory nucleus is not lateral but ventricular and that its extreme ventro-lateral border where the tela is attached is morphologically the dorsal border of the lateral brain wall. The lateral olfactory nucleus of Sheldon and especially the lobus pyriformis and nucleus taeniae, so far from being morphologically lateral are adjacent to the taenia fornicis and correspond to the dorso-medial border of the hemisphere in evaginated brains like that of the frog.

Third, the reason for thus ignoring the significance of eversion is found in the fact that the lateral olfactory tract is largely distributed to the everted ventricular region. This is therefore given the name of lateral olfactory nucleus. Now when the lateral olfactory nucleus in all other vertebrates is examined it is found that it never has the tela chorioidea attached to it and that it never is removed from the external surface of the brain and restricted to the ventricular surface. In all other vertebrates the lateral olfactory nucleus is separated from the tela chorioidea by at least the primordium hippocampi and it occupies a part of the true lateral or latero-basal surface of the telencephalon. The structure which Edinger, Goldstein, Kappers, Sheldon and others call the lateral olfactory nucleus in teleosts is a great mass oc-

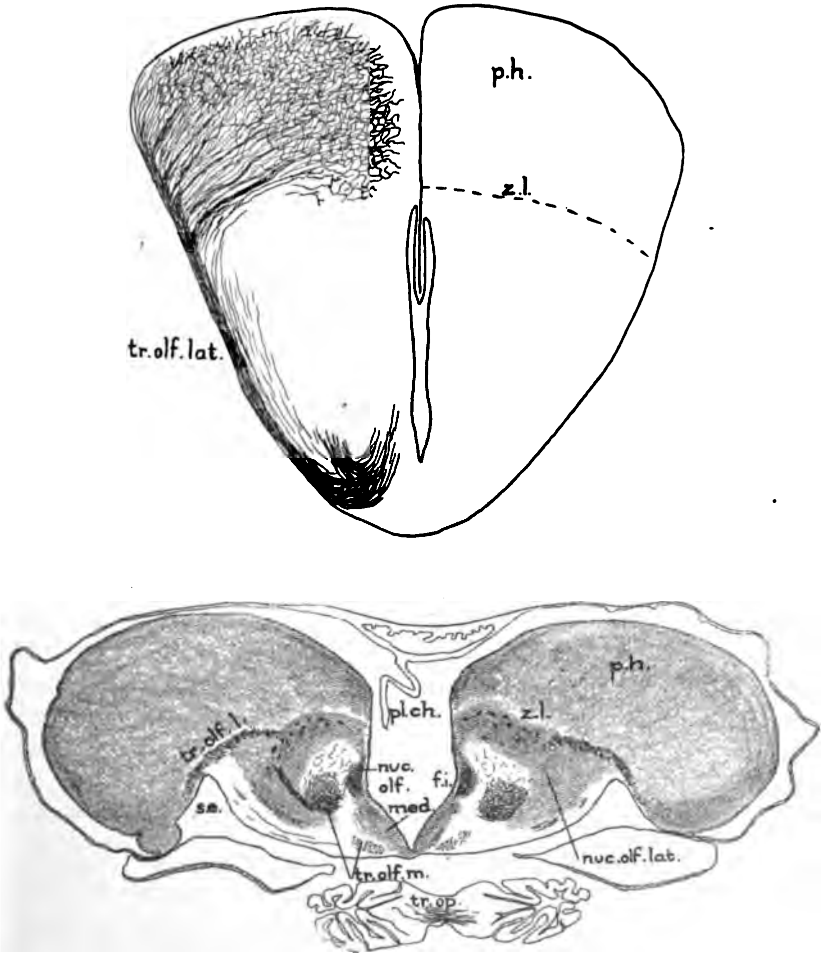


Fig. 1 Two figures for comparison of the transverse section of the telencephalon in front of the anterior commissure in a 15 mm. Amia (above) and in the adult. These figures show the actual change of form involved in eversion. It is the region dorsal to the zona limitans which is rolled and pushed outward, chiefly because of its own increase in volume. The same cause leads to the formation of the ventricular sulci. *f. i.*, groove leading to the interventricular foramen: *nuc. olf. med.* and *nuc. olf. lat.*, medial and lateral olfactory nuclei; *p.h.*, primordium hippocampi; *s.e.*, sulcus externus; *tr. olf. l.* and *tr. olf. m.*, lateral and medial olfactory tracts; *z.l.*, zona limitans.

cupying the ventricular surface and reaching the external surface only at its taenia-border.

The behavior of the lateral olfactory tract, however, does not compel us to identify this structure with the lateral olfactory nucleus, for two reasons. The lateral olfactory tract in selachians ends in large part in the primordium hippocampi, and in teleosts and ganoids a large part of the olfactory tract fibers which enter the so-called lateral olfactory nucleus come from the medial olfactory tract. On the other hand, in ganoids and teleosts a large part of the lateral olfactory tract ends in the latero-basal superficial area below the so-called sulcus or fovea endorhinalis. It is this area which really occupies the lateral surface of the forebrain as in all other vertebrates, which the writer believes is the lateral olfactory nucleus.

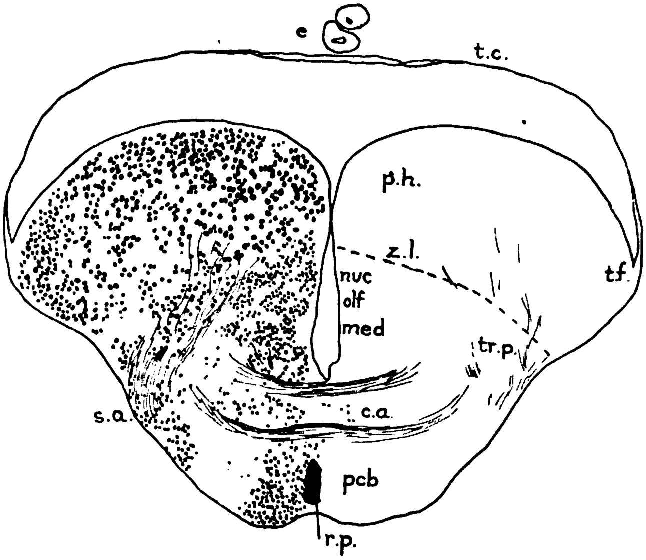


Fig. 2 A transverse section of the brain of Ameiurus of 19 mm., at the level of the anterior commissure. There is already slight eversion here as compared with the 15 mm. Amia and there is a broad shallow sulcus externus. Comparison of this with Sheldon's figures 38 and 56 will show that eversion in the teleost takes place in the same way as in Amia. *c.a.*, anterior commissure; *p.c.b.*, precommissural body; *r.p.*, preoptic recess; *s.a.*, somatic nucleus; *t.f.*, taenia fornicis; *tr.p.*, tractus pallii.

In all vertebrates the lateral olfactory nucleus is separated from the primordium pallii or pars pallialis hemisphaerii by a more or less conspicuous sulcus in the external surface of the brain. In petromyzonts this is the deep obliquely placed groove seen in the dorsal aspect between the roof of the evaginated hemisphere and the elevated wall of the telencephalon medium, which has been shown by the writer to be the homologue of the primordium hippocampi of other vertebrates. In selachians the primordium hippocampi is evaginated into the roof of the hemisphere, but is marked off from the lateral olfactory nucleus by a groove and a narrow cell-free zona limitans in the lateral wall. This zona limitans hippocampi extends along the lateral wall, over the olfactory peduncle and around the rostral wall to meet its fellow in the medial wall at the neuroporic recess. The parts of the wall below this zona limitans include the lateral and medial olfactory nuclei, tuberculum olfactorium, etc. In amphibians a similar zona limitans separates the pars pallialis from the pars sub-pallialis as Gaupp has shown. In reptiles and mammals and possibly even in amphibians the general or somatic cortex has pushed in between the lateral olfactory nucleus and the primordium hippocampi, so that the zona limitans, or the sulcus which marks it externally, becomes the dividing line between the lateral olfactory nucleus and the general cortex. This groove in mammals is the fissura rhinalis which bounds laterally the lateral olfactory nucleus and the pyriform lobe.

Earlier authors (C. L. and C. J. Herrick '91, Edinger '96, Goldstein '05, Kappers '06) compared the groove on the latero-basal aspect of the teleost brain with the fissura rhinalis of mammals. Goldstein ('05, p. 143) cites Edinger's view that this is one of the most important brain fissures and that the pallium is always to be found just lateral to it. Kappers and Theunissen ('08) present a new view, namely, that the teleostean sulcus represents the sulcus endorhinalis,—"weil sie innerhalb des secundären Riechgebietes liegt." This is the view now taken by C. J. Herrick and Sheldon. The difficulty with this view is that the sulcus in fishes does *not* lie within the secondary olfactory area. That the area above it, which is now under consideration, does receive secondary olfactory fibers is perfectly true. But so does the hippocampal formation throughout the vertebrate series. What has escaped the attention of these recent authors is the fact that the entrance of a large bundle of ascending fibers from the hypothalamus makes of this body something fundamentally different from the simple secondary olfactory centers. This has been fully set forth on page 525 of my recent paper on the forebrain of ganoids and teleosts.

Morphologically, however, it is clear that the sulcus endorhinalis of mammals does not correspond to the sulcus in question in fishes. The sulcus endorhinalis lies within the basal olfactory centers, between the lateral olfactory tract and the olfactory tubercle, and rostrad *ends upon the ventral surface of the olfactory peduncle.* In all fishes, amphibians and reptiles, the groove here under consideration extends along the lateral surface of the hemisphere, rises dorsally as it goes rostrad and *passes over the olfactory peduncle or olfactory bulb.* In this disposition the groove agrees with the fissura rhinalis of the mammalian brain and differs conspicuously from the sulcus endorhinalis. The writer is therefore convinced that the earlier view of Edinger, Kappers and Goldstein was correct.

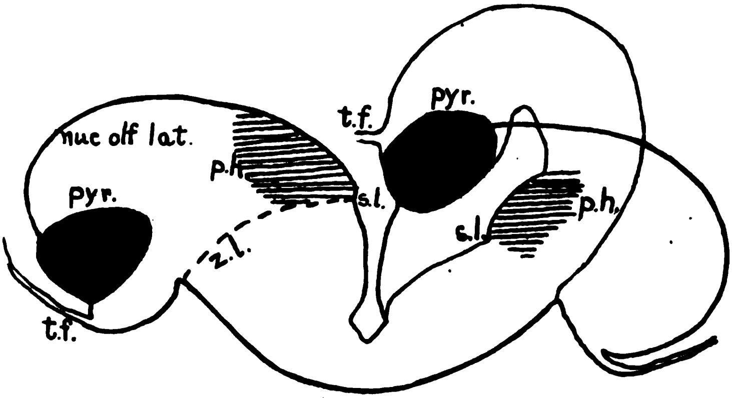
In my recent paper I gave to the broad groove in the teleost forebrain the descriptive name sulcus externus. This was done because I do not regard this broad groove as actually homologous with anything in the brains of other vertebrates. A zona limitans is usually recognizable in ganoids and teleosts and this is homologous with the zona limitans lateralis in selachians and amphibians. The *sulcus* limitans lateralis in selachians and amphibians is a very slight groove not comparable in size and form with the sulcus externus of the teleost brain. Moreover, this sulcus externus is not present in the young stages of either ganoids or teleosts. It appears only in later stages as the result of the process of eversion. It is formed by the actual folding outward of the whole thickness of the dorsal part of the lateral forebrain wall. It is only the line along which the cell-free zona limitans in the substance of the lateral lobe meets the lateral surface that can be compared with the sulcus limitans lateralis in selachians and amphibians. This line lies somewhere within the broad sulcus externus, but there appears to be considerable variation as to the form and position of this sulcus or fovea in adult ganoids and teleosts.

In the relation of the lateral olfactory tract to this sulcus there is further evidence for the homology of the sulcus limitans lateralis with the fissura rhinalis. The lateral olfactory tract runs longitudinally somewhat parallel with this sulcus, many of its fibers turning up into the primordium hippocampi. When the eversion of the brain wall is taken into account it is seen that the morphological position of the lateral tract is medio-basal to the sulcus. This is clear from Goldstein's figure 4 and Sheldon's figure 24, and is still more clear in the simpler ganoids and in young ganoids and teleosts. In Acipenser of 30 cm. where the eversion is slight, the lateral olfactory tract lies wholly basal to the zona limitans. In adult Amia ('11 b, figs. 5, 6) the lateral olfactory tract lies partly in the zona limitans and partly basal to it. In Polyodon ('11 b, figs. 55, 56) the lateral tract evidently lies wholly medio-basal to the zona limitans. The same condition prevails in the selachians; and in mammals, where the somatic cortex has pushed in between the olfactory tract and the hippocampus, the lateral tract lies in the same relation to the fissura rhinalis as it holds to the zona limitans lateralis in the fishes.

The above considerations have led the writer to the conclusion that the area seen from the ventricular surface in teleosts between the sulcus limitans hippocampi and the line of attachment of the tela chorioidea is the primordium hippocampi as it is in cyclostomes and selachians (and also in amphibians and reptiles except that the somatic cortex pushes into this area). The entire region has essentially the same structure and fiber connections throughout, although in adult teleosts the great increase in volume is accompanied by eversion and by the formation of new sulci such as the sulcus ypsiliformis. This has been accompanied by the segregation of gray masses and fiber bundles which Sheldon and others have described as distinct centers and tracts.

This view carries with it the interpretation of the lateral superficial part of the olfactory lobe rostral to the anterior commissure as the lateral olfactory nucleus. This area is included by Sheldon in his nucleus medianus septi, but it must be observed that it belongs to the lateral wall of the brain and has nothing to do with the septal region. In ganoids it is largely separted from the nucleus medianus septi by that part of the olfactory bulb which is called nucleus olfactorius anterior ('11 b, figs. 5, 70). This true lateral olfactory nucleus extends caudad only about to the level of the anterior commissure where it meets with the somatic area. In selachians a well developed correlating tract has been found between the two centers and in higher vertebrates a part of this somatic area doubtless remains in connection with the lateral olfactory nucleus to constitute the pyriform lobe.

Dr. Sheldon locates the lobus pyriformis in the extreme lateral part of the lateral lobe to which the tela chorioidea is attached. Upon the view of eversion of the teleost forebrain held by Mrs. Gage, Kappers, the writer and others, this part of the lateral lobe corresponds to the medio-dorsal border in evaginated brains. The region which Sheldon has called the primordium hippocampi, on the other hand, corresponds to a portion of the lateral wall of the evaginated brains. This is illustrated in figure 3 which shows at a glance how untenable this interpretation is. Dr. Sheldon has seen the weakness of these homologies and has suggested that the form of the teleost forebrain is due not to a process of eversion such as Mrs. Gage set forth but to a wandering and shifting of the relative position of the gray masses (Sheldon '12, pp. 244–245). No facts are given upon which this hypothesis of a modified eversion is based and the writer has seen nothing in his study of larval stages of ganoids and teleosts to give support to it. The process of eversion in teleosts seems to the writer to consist of a turning outward together with some plastic pushing or shifting laterad of the whole mass of the lateral wall dorsal to the zona limitans. The turning outward carries with it the attachment of the tela chorioidea; and the dorsal border—taenia fornicis—in ganoids and teleosts is the same as in all other vertebrates, but is carried far latero-ventrally by eversion. The writer fails to see any ground upon which we may expect to find the lobus pyriformis in any vertebrate located adjacent to the taenia fornicis.



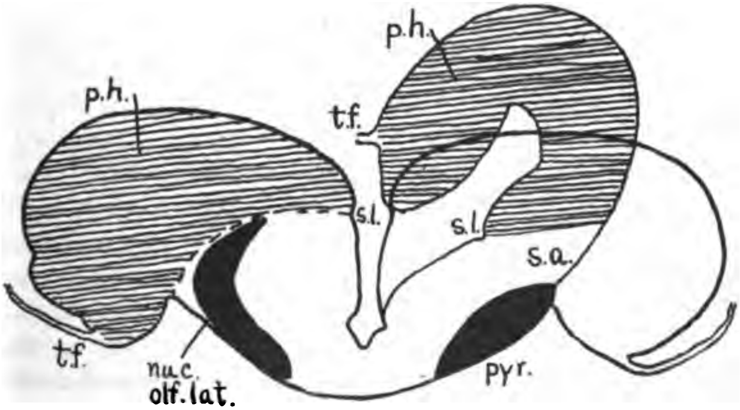


Fig. 3 Two diagrams based upon transverse sections at the level of the anterior commissure of Amia, to illustrate the conceptions of the relations of the primordium hippocampi held by Dr. Sheldon and by the writer. In both diagrams the form of cross section of the hemisphere of an amphibian is superimposed upon the right half of the Amia section. On the left in the upper diagram the position of the pyriform lobe and primordium hippocampi is shown as Sheldon has located them. On the right the position of these centers is shown as they would come to lie in a brain which was evaginated. In the lower diagram the position of these centers is shown as they are understood by the writer, *pyr.*, pyriform lobe; *s.l.*, sulcus limitans hippocampi. *s.a.* in this figure indicates the region where the somatic nucleus pushes between the basal olfactory centers and the primordium hippocampi to give rise to the general cortex.

As to the homology of the nucleus olfactorius dorsalis of the carp with the primordium hippocampi of the frog, Sheldon admits that there are few points of resemblance (p. 246). He concludes (p. 247):

> The materials found in the amphibian primordium hippocampi are not completely separated in the teleosts from the other elements of the secondary olfactory nucleus, being represented chiefly in the nucleus olfactorius dorsalis or primordium hippocampi and to a less degree perhaps in the nucleus olfactorius lateralis and nucleus pyriformis.

This approaches closely the view of the present writer. If this whole structure dorsal to the zona limitans is considered to be primordium hippocampi, its resemblance to that of the frog and selachians, in position, structure and connections, is clear.

In this connection Sheldon compares his sulcus limitans telencephali with the fissura limitans hippocampi of Herrick in the frog (fissura arcuata of Gaupp), although he states that the homology is incomplete. Indeed it is difficult to see what could lead the author to make any comparison between the two. The teleostean sulcus limitans telencephali lies in the ventricular surface of the *lateral* brain wall, while the fissura limitans hippocampi of Herrick lies in the *medial* wall of evaginated brains. The teleostean sulcus clearly is identical with the sulcus limitans lateralis of the frog's brain, and a fissura limitans hippocampi (Herrick) cannot be present or be conceived of in an unevaginated or everted brain. Such a sulcus can be present only in those forms in which evagination has carried a part of the medial olfactory nucleus out into the medial wall of the hemisphere between the olfactory bulb and the lamina terminalis. Here the sulcus in question separates the medial olfactory nucleus from the primordium hippocampi which occupies the roof. This sulcus I have called the *medial* sulcus limitans hippocampi, while the *lateral* sulcus limitans hippocampi separates the primordium hippocampi from the lateral olfactory nucleus in the lateral wall, (Johnston '11 a). In ganoids and teleosts nothing is evaginated but the olfactory bulb, there is no roof containing a primordium hippocampi and there is no medial olfactory nucleus in the medial wall of an evaginated hemisphere. Therefore there is no medial sulcus limitans hippocampi.

Sheldon describes a nucleus commissuralis lateralis and a nucleus entopeduncularis which together seem to correspond to the writer's somatic nucleus. I see no reason for including the palaeostriatum, nucleus taeniae or the nucleus intermedius in the somatic area, as suggested by Sheldon. It is not clear what different writers mean by 'nucleus taeniae.' Sheldon's nucleus taeniae appears to be the same as Edinger's and Kappers', olfactory in function. Goldstein's nucleus taeniae is described as non-olfactory in function and it is probable that the body which he described belongs to the writer's somatic nucleus. Goldstein's nucleus entopeduncularis lies ventrally close to the chiasma and Sheldon can scarcely have identified it correctly in the carp. In Ameiurus there is a nucleus entopeduncularis independent of the somatic nucleus and ventral to it. This has apparently not been seen in the carp, unless it is included in the complex nucleus praeopticus. Sheldon's nucleus entopeduncularis is undoubtedly identical with the caudal part of my somatic nucleus.

The theoretical part of Dr. Sheldon's paper is a restatement of Herrick's theory regarding longitudinal columns in the telencephalon. The writer is unable to see that this theory has any relation to the facts set forth in the descriptive part of Sheldon's paper. The diagrams on plate 35 are evidently intended to illustrate this theory but not to give any facts for its support. Nothing is said in the paper to indicate the grounds on which the peculiar shifting about of the columns 1, 2 and 3 in these diagrams is based. If the writer's view is correct, the primordium hippocampi includes all or nearly all of the territory represented by the numerals *1*, *2* and *3* in figures 132, 133, 134. The somatic nucleus is not taken into account at all in these diagrams. In the text it is stated (p. 243), "the nucleus entopeduncularis [my somatic nucleus] probably belongs to the same column as the pars ventralis thalami, the pars ventro-lateralis of Herrick, which expands rostrally to form the palaeostriatum." That this view is untenable is clear from the simple fact that this nucleus is directly continuous caudally with the optic centers and closely related to the other sensory centers which lie in Herrick's pars dorsalis thalami. The description of the palaeostriatum given by Sheldon shows that it has the same structure and connections as his lateral olfactory nucleus. I have specifically included the corresponding region in ganoids in my primordium hippocampi. It seems, then, that there is nothing in the telencephalon that may be regarded as the continuation of Herrick's column 3 of the diencephalon.

I have elsewhere ('11 b) criticized Herrick's schema because he sees the telencephalic continuation of the somatic sensory column of the diencephalon ('pars dorsalis thalami') in a structure of wholly different functional significance, namely, the lateral olfactory nucleus. Here in fishes the impropriety of this becomes evident from the fact that the pars dorsalis thalami has an actual structural continuation in the form of the somatic sensory nucleus of the telencephalon. Essentially the same condition prevails in all vertebrates.

Another difficulty with the application of Herrick's schema to the teleost brain is seen in the assignment of the substance adjacent to the taenia fornicis to column *2*, while the primordium hippocampi which is the telencephalic continuation of the most dorsal column *1* is placed in the lateral wall morphologically ventral to column *2*.

Herrick's schema when applied to the teleost brain appears artificial and inconsistent with the described facts.

Dr. Sheldon uses the term 'hemisphere' for the lateral lobes which are not evaginated. Professor Herrick ('10) has advocated the use of the term for the evaginated part of the telencephalon as distinguished from the telencephalon medium. The writer ('12 b) accepts this, and it is unfortunate that the same usage should not have been followed in all papers coming from Professor Herrick's laboratory.

It is thought that the comments in the foregoing paper will be clear to anyone who has before him the figures in the papers under discussion. The literature cited is also listed in those papers.

Reprinted from THE JOURNAL OF COMPARATIVE NEUROLOGY, VOL. 22, No. 4
August, 1912

THE TELENCEPHALON IN CYCLOSTOMES[1]

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From The Institute of Anatomy, University of Minnesota

FORTY-ONE FIGURES

The telencephalon of cyclostomes presents in many ways more primitive conditions than are known in other vertebrates. Cyclostomes are therfore important in the effort to trace the phylogeny of various structures in the cerebral hemispheres. The discussions regarding the interpretation of the lateral lobes, the pallium and the ventricles (Ahlborn, Rabl-Rückhard, Studnicka, Edinger) together with the contributions by Sterzi ('09) and the writer ('02a), have made clear the significance of most parts of the telencephalon at least in Petromyzonts. The lateral lobes are true hemispheres,[2] containing lateral ventricles connected with the third ventricle by wide interventricular foramina. The hemispheres are pushed back against the sides of the diencephalon by pressure from the buccal apparatus. The bulbar formation occupies the broad rostral wall of the hemisphere and does not project forward as a bulbus olfactorius. In addition to this bulbar formation the hemisphere walls include secondary olfactory centers and a basal-central area heretofore known as corpus striatum. The bulbar formation and secondary olfactory centers are separated by a groove which represents the olfactory peduncle.

The anterior portion of the third ventricle is closed above and rostrally by a membrane which is thickened in two places by commissures (figs. 5. 6). Rostral to the extraordinarily thick chiasma ridge the lamina terminalis extends from the preoptic recess to the recessus neuroporicus, rostral to the interventricular foramen. The position of the recessus neuroporicus was first clearly established by Sterzi ('07). The lamina terminalis is thickened by the anterior commissure. Above the recessus neuroporicus the roof of the ventricle is thickened by the so-called dorsal olfactory commissure or decussation. This thickened lamina should be called the lamina supraneuroporica (Burckhardt '94 b, '94 c, '07, Johnston, '11 b). A short distance caudal to this lamina occurs a small fold in the membranous roof which was shown by Sterzi ('07) to be the velum transversum. Caudal to this a long dorsal sac extends to the superior commissure and habenular bodies. This dorsal sac is covered dorsally by the parapineal body and epiphysis, which depress the sac in various degrees.

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[1] Neurological Studies from the Institute of Anatomy, University of Minnesota, No. 16.

[2] The writer here uses the term hemisphere as synonymous with the lateral lobe or evagination of the telencephalon, accepting in this the suggestion of Professor Herrick ('10, p. 492).

Upon the general morphology of the petromyzont telencephalon thus far there is general agreement among workers. There are, however, disputed questions regarding the relations of the telencephalon and diencephalon, and the location of the primordium of the cortical area of higher vertebrates.

The writer has reviewed the preparations previously studied and has examined new preparations of the same and other species of petromyzonts. The writer wishes to express his sincere thanks to Professor Gage for a very generous supply of ammocoetes and adults of both Lampetra and Petromyzon dorsatus. Other specimens of Lampetra have been obtained from Professor Reighard's laboratory both while the writer was located there and later through the kind assistance of Dr. L. J. Cole. The writer is deeply indebted to Professor Charles Brookover for the loan of the series of sections of Ichthyomyzon which is described beyond. Thanks are also due to Professor Reighard for the loan of a series of sections of a new dwarf lamprey not yet described. Through the kindness of Mr. W. F. Allen of this laboratory I have had the opportunity to section and study several stages of the ammocoetes of the Pacific coast lamprey, Entosphenus.

The di-telencephalic boundary

The writer has attempted ('09) an accurate definition of the boundary in question, upon the basis of selachian, amphibian, avian and mammalian embryos. The result was to show that the optic chiasma belongs to the telencephalon, the boundary being defined by the velum transversum above and the caudal surface of the chiasma ridge below. It was shown that the telencephalon includes, in addition to the hemispheres, a median portion surrounding the rostral part of the third ventricle. The floor of this telencephalon medium is occupied by the optic chiasma. Its roof (roof plate of His) is made up of the lamina terminalis, lamina supraneuroporica and tela chorioidea. The telencephalon constitutes a complete brain ring or segment as His contended, although shorter than His thought. The hemispheres are lateral evaginations of this telencephalon medium. In the series of vertebrates, as in the stages of the ontogeny, a progressively larger part of the telencephalon is evaginated into the hemispheres, until in man only the chiasma ridge and the small region between it and the lamina terminalis remains as the telencephalon medium.

This definition of the di-telencephalic boundary has been accepted by Herrick ('10), Kappers and Carpenter ('11), and others, and its substantial correctness will be assumed for the purposes of this paper. If the di-telencephalic boundary is to be determined in cyclostomes in the same way as in other vertebrates, it becomes necessary only to describe the velum transversum in cyclostomes accurately and completely, since the chiasma ridge is already well understood.

The recognition by Sterzi of the small fold behind the dorsal decussation as the homologue of the velum transversum of higher forms was a valuable contribution to the interpretation of the cyclostome brain. The velum was recognized, however, only in median sections and the postition of the velum in the median plane does not define the boundary between the telencephalon and diencephalon. The writer has shown at length elsewhere ('11 a, '11 b) the errors and inconsistencies which arise from taking into account only the position of the velum in the median plane. It is the point of attachment of the velum to the massive walls which determines the boundary between telencephalon and diencephalon. The velum transversum is a fold of the tela chorioidea having the form of an arch whose pillars rest on the massive lateral walls. This point of attachment is the meeting place of the taenia thalami and taenia fornicis. The position of the velar arch in the median plane depends upon the form of the tela chorioidea of the third ventricle as affected by the general form of the brain and the pressure or traction exerted upon the tela by surrounding structures. Thus in the selachian brain the velum is nearly transverse and vertical in position (fig. 1) and is attached to the lateral walls just in front of the habenular bodies. In certain ganoids, on the other hand, the velum is a very deep fold which is inclined forward at an angle greater than 45 degrees. The pillars of the velum are attached to the lateral walls just rostral to the habenular bodies exactly as in selachians (fig. 2). The point of attachment of the velum to the lateral walls may be more sharply defined with reference to the internal structure of the massive wall. It is just at the point of attachment of the velum that the several categories of fibers which make up the stria medullaris converge into a compact bundle to ascend to the habenular nucleus and commissure (commissura superior Osborn). In amphibians and reptiles this portion of the lateral wall, to which the velum is attached and which is traversed by the stria medullaris, Herrick ('10, p. 419) has called the eminentia thalami. This low eminence is clearly seen in figures 1 and 2, but is not lettered.

A careful study of the velum transversum in Lampetra shows that it is attached as in fishes and amphibians immediately rostral to the habenular bodies and that its point of attachment is the dorsal border of the eminentia thalami.

As is well known, the right habenular body is very much larger than the left, and each is bounded ventrally by a deep groove, the sub-habenular sulcus (figs 3, 5, 6). At its rostral end this deep sulcus leads into the dorsal sac. Here it meets with a vertical sulcus which descends in the brain wall and curves forward to enter the interventricular foramen (figs. 5, 6, sulcus limitans hippocampi). The common space formed by the union of these two deep sulci where they join in the caudal and lateral angle of the dorsal sac is a very deep and narrow cleft which may be called the recessus praehabenularis (figs. 14, 18). This deep recess has been formed probably by the crowding together of the brain due to pressure from in front. Below the habenular body two nearly vertical ridges are seen (figs. 5, 6) in the side wall of the thalamus. The more caudal one is occupied by the tractus habenulo-peduncularis (figs. 21, 22). It is much more prominent on the right, owing to the greater size of the nucleus habenulae and the fiber tract on the right side. The more cephalic ridge is about equally developed on the two sides and contains the stria medullaris (figs. 20, 21). This I shall call the eminentia thalami. It is bounded in front by the sulcus limitans hippocampi and extends up in the recessus praehabenularis as a narrow ridge (figs. 20, 26, 27). The groove separating the two ridges corresponds to the sulcus *b* of the selachian brain (Johnston '11 a) and to the sulcus diencephalicus medius of Herrick ('10) in amphibians.

The dorsal ridge in front of the habenular body is large in Lampetra and presents a slight but distinct eversion similar to that in the teleost brain (figs. 7, 30, 31). To this ridge the writer formerly ('02 a) gave the name epistriatum. In anticipation of the results of the following pages, we may call it here the primordium hippocampi. It extends forward over the interventricular foramen where it becomes continuous with the roof of the lateral evagination.

In order to determine whether this ridge belongs to the telencephalon or diencephalon we must discover the point of attachment of the velum transversum to the massive walls. This point is difficult to determine in most petromyzonts because the velum is very rudimentary and is recognizable only near the median line. This is true of the adults of Petromyzon dorsatus and Lampetra which the writer has examined, of Ichthyomyzon studied by Herrick and apparently of the forms studied by European authors. In the ammocoetes of Petromyzon dorsatus, however, the velum is better developed and appears as a fold which extends across the whole width of the tela so that the attachment to the massive walls can be determined.

In order to demonstrate the pillars of the velar arch in this form the writer has made a plate reconstruction of the dorsal part of the telencephalon and diencephalon. Owing to the compression of the dorsal sac by the epiphysis and parapineal body the velum shows to best advantage in sagittal sections and the reconstruction was made from these (fig. 8). The model was made from the left side of the brain and included the recessus neuroporicus in front and a part of the nucleus habenulae behind. In order to see as much of the velum as possible the model has been drawn from a medio-ventro-caudal direction. This figure should be compared with figure 3, which shows the left half of the forebrain from a model of another specimen of Petromyzon dorsatus (ammocoetes). The cut surface along the upper border of the figure corresponds very nearly to the median sagittal plane. In this plane is seen the fold described by Sterzi as the velum transversum. Extending caudo-laterally from this above the primordium hippocampi is a fold of the tela which continues without interruption into the extreme caudo-lateral angle of the dorsal portion of the ventricle. The deep cleft in which the velum is seen in the figure is the recessus praehabenularis described above. In the depth of this recess the lateral pillar of the velar arch is attached to a small ridge which is identified as the eminentia thalami by the presence in it of the stria medullaris. As the model is viewed from an unusual angle, the relations will perhaps be more clear by comparison with figures 23 to 27, which represent five sections of the series from which the model was constructed. The relations of the recessus praehabenularis and of the eminentia thalami in Lampetra are shown in figures 14 to 22. Here the sulcus praehabenularis is narrower than in the ammocoetes because of the enlargement and crowding of the surrounding parts. The disposition of the velum transversum in Petromyzon dorsatus is essentially the same as that in ganoids and teleosts. The velum is not only inclined far forward as in the latter fishes, but owing to the depression of the dorsal sac by the overlying epiphysis, its middle part is pressed down far below the plane of attachment of its pillars. The whole course of the velum is diagrammatically represented in figure 28 as it might be seen in the dorsal aspect of the brain. As indicated in this figure, that portion of the dorsal ventricular space which lies above the velum transversum is properly called the dorsal sac, while the portion below the velum belongs to the telencephalic portion of the third ventricle.

This description of the velum transversum makes it possible to define clearly the boundary between the telencephalon and diencephalon. It is marked, as shown in figures 5 and 6, by a line running from the attachment of the velum transversum upon the dorso-rostral border of the eminentia thalami to the caudal surface of the chiasma ridge.

The ventricular sulci in diencephalon and telencephalon

Attention has been called above to the sulcus limitans hippocampi, the dorsal or sub-habenular sulcus and the sulcus *b* or sulcus medius. In figure 5 are to be seen three other important ventricular grooves, the sulcus limitans of His, the sulcus hypothalamicus and a sulcus connecting the recessus praeopticus with the foramen interventriculare. The presence of the last named in embryos and adults of other classes of vertebrates has been pointed out ('11 a). The further study of slightly evaginated brains (cyclostomes, ganoids and teleosts) makes it necessary to withdraw the view stated earlier (in '11 a, p. 45) that the sulcus arising in the preoptic recess is the continuation of the sulcus limitans hippocampi.

The sulcus hypothalamicus is seen a short distance behind the interventricular foramen diverging ventrally from the sulcus limitans hippocampi. It grows deeper and descends into the hypothalamus as a crescentic groove. Discussion of the question whether this sulcus or any part of it is comparable to the sulcus hypothalamicus of the human brain is reserved. The name is used here in a purely descriptive sense and is evidently appropriate. The sulcus is the same as Herrick's sulcus diencephalicus ventralis. This name has not been adopted because the sulcus in all lower vertebrates is a transverse rather than a longitudinal sulcus.

The sulcus limitans of His traverses the midbrain in the same position as in other vertebrates and meets the sulcus hypothalamicus over and in front of the tuberculum posterius. It can not be traced forward to the preoptic recess, apparently owing to the great prominence of the chiasma-ridge and the supra-optic nucleus.

Professor Herrick has given an account of the sulci in Ichthyomyzon which differs from the above account in important respects. With Professor Herrick's kind permission I reproduce his figure 73 as figure 29 of this paper. Comparison of this with my figure 5 representing the model of the Lampetra forebrain shows that Herrick has given the name sulcus diencephalicus medius to a part of my sulcus hypothalamicus and that he has described the lower end of this sulcus hypothalamicus as a separate sulcus under the name of the sulcus diencephalicus ventralis. He recognizes also a sulcus subhabenularis and sulcus diencephalicus dorsalis.

As it was impossible to harmonize this description with the condition in Lampetra, I have secured for study at Professor Herrick's suggestion the identical series of sections from which his drawings were made. The sections are fifteen microns thick and with the exception of a single broken section the series is perfect. A model has been made at a magnification of 100 diameters, the right half of which is drawn from the ventricular surface in figure 6. The model when finished was a trifle longer than it should be, in the proportion of 94 to 90.

Referring to Herrick's figures 74 to 81, it should be noted that Professor Herrick viewed the sections from in front, so that the right side of the brain appears in the left side of his figures. Thus the right nucleus habenulae, which is the larger, appears on the left side in figures 80 and 81. From this it follows that the reconstruction in figure 73 (figure 29 of this paper) represents the ventricular surface of the right half of the brain as if it were the left half. This is mentioned only to show that the model figured is properly to be compared directly with Herrick's reconstruction.

The model shows the following points. The primordium hippocampi is not so large as in Lampetra but has the same form and relations. The nucleus habenulae projects rostrad somewhat over the primordium hippocampi. The sulcus limitans hippocampi and sulcus hypothalamicus very closely resemble those in Lampetra. The eminentia thalami is better marked than in Lampetra, being a prominent ridge near the nucleus habenulae.

Behind the eminentia thalami is a ridge extending from the nucleus habenulae to the interpeduncular region and occupied by the tractus habenulo-peduncularis. Between this ridge and the eminentia thalami is a sulcus diencephalicus medius which is somewhat more regular than in Lampetra. The sulcus limitans of His is somewhat less pronounced than in Lampetra.

Professor Herrick thought that he found in Ichthyomyzon conditions which supported his theory regarding the division of the diencephalon and the telencephalon of amphibians into four longitudinal columns. In amphibians (Herrick, '10, p. 419) the sulcus medius forms the dorsal boundary of the eminentia thalami and runs caudally toward the tuberculum posterius. The statement that this sulcus and the sulcus ventralis 'converge anteriorly to the interventricular foramen' is evidently without foundation, since the sulcus medius is situated dorso-caudal to the eminentia thalami and the velum transversum, and can not reach the interventricular foramen. In the amphibians studied by the writer (Amblystoma, Necturus, Cryptobranchus, Rana, Bufo) the sulcus medius runs up into the dorsal sac and has no relation to the interventricular foramen. This fact is clearly shown also in Herrick's figures 5, 18, 19, 22, 33 and 34.

In Herrick's Ichthyomyzon figures 78, 79 and 80, the *s.m.* corresponds to my sulcus limitans hippocampi, while in figure 81 and on the right side of figure 80, *s.m.* is the sulcus hypothalamicus. Consistent with his indentification of this with the sulcus medius, Herrick labels the area below it as the pars ventralis thalami and compares it with the eminentia thalami of amphibians ('10, p. 471). This interpretation is obviously untenable, since the eminentia thalami of amphibians is caudo-dorsal to the interventricular foramen, immediately adjacent to the nucleus habenulae, is bounded below by the sulcus diencephalicus ventralis and is traversed by the compact stria medullaris just before this bundle enters the nucleus habenulae. See Herrick's figures 17– to 22. The true position of the eminentia thalami in Ichthyomyzon is clear from Herrick's figures 80 and 81 in which the compact stria medullaris is about to enter the nucleus habenulae. These figures show that the eminentia thalami lies as in amphibians at the dorsal border of the brain near the nucleus habenulae. This is more clear from my figure 9 which is drawn from a section between the two drawn in Herrick's figures 80 and 81. In this section the stria medullaris is seen in the eminentia thalami. In Herrick's figure 81 the stria medullaris has bent laterad into the outer portion of the nucleus habenulae and the tractus habenulopeduncularis is coming down near the ventricle. Having thus identified the eminentia thalami, it is evident that the groove in Ichthyomyzon which corresponds to the sulcus medius of amphibians is the groove so lettered in figure 6.

Professor Herrick has completely overloooked the greater part of the sulcus limitans hippocampi in Ichthyomyzon, probably because the greater part of its course lies more or less parallel with the plane of the transverse sections. For the same reason he failed to recognize that his *s.m.* and *s.v.* were the two ends of one and the same crescentic groove. The model clearly shows the true relations in both cases, and readily explains how natural Professor Herrick's interpretation was in the absence of models.

In dealing with the relations of the telencephalon and diencephalon in the dorsal region, Professor Herrick ('10, pp. 473–4) says,

> On account of the very small degree of evagination of the cerebral hemisphere in cyclostomes the di-telencephalic fissure is shallow and the pars dorsalis thalami passes over without interruption into the lateral wall (lobus olfactorius) of the hemisphere. Moreover this fissure does not extend upward to the mid-dorsal line and thus the dorso-median ridge is able to pass continuously from one segment to the other. In higher vertebrates this fissure extends dorsally up to the site of the velum transversum and it is so deep as to interrupt the continuity of both the ridge and all other massive tissue of the pars dorsalis thalami with their telencephalic representatives.

It is necessary to define clearly what is meant by the di-telencephalic fissure. In the human and mammalian brain there is a great groove or fissure between the posterior part of the hemisphere and the thalamus, midbrain and cerebellum. Near the bottom of this is the chorioidal fissure of the hemisphere. Dorsally the fissures of the two sides join in the great longitudinal fissure. Taken together these constitute in a true sense a margiginal or limiting fissure of the hemispheres. It owes its existence to the fact that the evagination of the hemisphere causes an angle or fold between its wall and the wall of the brain stem. The whole fissure may therefore be referred to under the descriptive term, stem-hemisphere fissure. When lower vertebrates are examined it is seen that the stem-hemisphere fissure is well-marked in reptiles and amphibians, but in true fishes is only a broad shallow groove or constriction. In Petromyzonts, one of the most striking features is the sharp separation between hemisphere and brain stem (fig. 7). In a previous paper ('09) the writer has shown that this is not the line of division between telencephalon and diencephalon. The groove seen in figure 7 running from near the median line in front outward and backward is the stem-hemisphere fissure. It marks the boundary between the hemisphere and the telencephalon medium, not only in cyclostomes but in all classes of vertebrates. When the hemispheres expand and lie apposed to each other in the median plane, this forms the great longitudinal fissure and its lateral extension between the posterior pole of the hemisphere and the brain stem.

Obviously this stem-hemisphere fissure can not be called a di-telencephalic fissure. The writer has suggested ('09, p. 516) that the di-telencephalic fissure owes its origin to the withdrawal of tissue to form the optic vesicle. In this Professor Herrick concurs ('10, p. 467). The di-telencephalic fissure lies at the junction of the telencephalon medium and diencephalon, while the hemisphere evagination takes place some distance farther rostrad, and the two are entirely independent. That this is so is perfectly clear from cyclostomes, selachians and other fishes. The evagination of the hemispheres has, therefore, nothing to do with the di-telencephalic fissure or the continuity of diencephalon and telencephalon. Further, the di-telencephalic fissure is dorsal in position from the start and does not extend farther dorsally in higher vertebrates. What does happen is that in higher vertebrates more and more of the telencephalon medium comes to be evaginated into the hemispheres until the stem-hemisphere fissure gradually approaches the di-telencephalic fissure.

In cyclostomes the di-telencephalic fissure is as clearly present as in other vertebrates. It is marked by the eminentia thalami to which the velum transversum is attached. In the ammocoetes of Petromyzon dorsatus, at least, the di-telencephalic boundary is further marked by an external groove (fig. 4). Professor Herrick is in error in his speculations regarding the continuity of the dorsal part of the thalamus and the telencephalon in cyclostomes (pp. 474 and 477–8). The di-telencephalic fissure is slightly masked in cyclostomes because of the crowding back of the telencephalon against the diencephalon but the sulcus medius comes to the dorsal border here precisely as in amphibians and if the dorsal column is interrupted by the di-telencephalic fissure in amphibians, it is interrupted in just the same way in cyclostomes.

Herrick states (p. 472) that he has indicated in figure 73 by a dotted line (*s.d.*) 'a somewhat arbitrary boundary' between his dorso-median ridge (my primordium hippocampi) and his pars dorsalis thalami. This dotted line does not correspond to the sulcus limitans hippocampi or to any thing that I can find in Lampetra, Petromyzon dorsatus, Entosphenus or Ichthyomyzon. The dotted line is lettered sulcus diencephalicus dorsalis while in the text (p. 470) the dorsal is spoken of as synonymous with the sub-habenular sulcus. In amphibians (Herrick '10, 431 and fig. 22) the subhabenular and dorsal sulci are distinct but are regarded as two parts of a sulcus which separates the epithalamus from the dorsal part of the thalamus. In amphibians the dorsal is the more caudal segment of the common sulcus. In the Ichthyomyzon diagram the positions are reversed. In amphibians neither of these sulci has even a remote relation with the interventricular foramen, and both lie wholly within the diencephalon. In the Ichthyomyzon diagram the line *s.d.* is connected with the foramen and lies wholly within the telencephalon. This arbitrary line in Ichthyomyzon has therefore no relation to the sulcus diencephalicus dorsalis of amphibians.

Herrick regards the groove which extends rostrad from the foramen as a continuation of the sulcus diencephalicus dorsalis. In this position there are two grooves in Ichthyomyzon and in Lampetra. One extends forward from the dorsal angle of the foramen, the other from the ventral angle, and the two converge into the neuroporic recess. The lower one of these grooves is the one designated by Herrick as the telencephalic extension of the sulcus dorsalis (Herrick '10, figs. 75, 76). The dorso-median ridge (my promordium hippocampi) ends rostrally in the sections which contain the so-called dorsal olfactory commissure. As seen in the model, it is very abruptly reduced in dorso-ventral thickness at the foramen and extends over the foramen only as a slender strand of cells (which appears in Herrick's fig. 77) in close connection with the commissure. Rostral to the foramen this slender ridge disappears entirely and the larger ridge which Herrick calls the dorso-median ridge in his figures 75 and 76 contains glomeruli and olfactory fibers and belongs to the formatio bulbaris. Of the two sulci extending rostrally from the foramen, the upper one separates the dorso-median ridge (primordium hippocampi) from the formatio bulbaris, the lower one separates the formatio bulbaris from the medial olfactory nucleus.

In the ammocoetes of Petromyzon dorsatus (figs. 3, 8) there is only one groove extending from the foramen to the neuroporic recess. This sulcus separates the primordium hippocampi from the medial olfactory nucleus and there is no formatio bulbaris in this position . Lampetra presents an intermediate condition. The two grooves are nearer together and the area of formatio bulbaris which abuts on the ventricle is less than in Ichythyomyzon. These facts show that in Ichthyomyzon and Lampetra the evagination of the hemisphere has not completely carried out the formatio bulbaris, but that a part of this formation remains in the telencephalon medium and forms part of the wall of the median ventricle rostral to the foramen. In Petromyzon dorsatus the evagination of the formatio bulbaris is complete. Consequently, the two sulci in Ichthyomyzon and Lampetra may be regarded as merged into one sulcus in Petromyzon dorsatus. This one sulcus begins in the neuroporic recess and passes into the rostral wall of the lateral ventricle, separating the primordium hippocampi from the medial olfactory nucleus. The position of this sulcus is the same as that of the medial zona limitans (hippocampi) in selachians (Johnston, '11 a).

The brain of Professor Reighard's dwarf lamprey presents larger hemispheres with wider lateral ventricles than I have seen in any other petromyzont. In the form and size of the primordium hippocampi and in the disposition of the ventricular sulci it agrees well with Lampetra.

The primordium hippocampi

The description of the ventricular sulci has made clear the definite ventral boundary of this body on the ventricular side. Externally a very deep groove separates it from the caudal pole of the hemisphere (fig. 7). At the interventricular foramen the primordium hippocampi bends through the roof of the foramen to become directly continuous with the roof of the hemisphere. Caudally, the sulcus limitans separates this body sharply from the eminentia thalami and nucleus habenulae internally, but on the external surface there is no visible boundary in adult Lampetra. In the ammocoetes of Petromyzon dorsatus the external surface shows a vertical groove which marks the caudal boundary of the telencephalon (fig. 4).

The neurones of this body belong to a special type which is found nowhere else in the brain of Lampetra. The same type of neurone is characteristic of the primordium hippocampi of ganoids and amphibians. As far as the writer's studies have gone, these neurones are as truly characteristic of the primordium hippocampi as are the Purkinje cells of the cerebellum, and are much more highly differentiated in cyclostomes than are the Purkinje cells.

In a fomer paper ('02 a, p. 40) these neurones as they appear in Golgi preparations were discribed as follows:

> The cells of the epistriatum are arranged in two to four rows adjoining the cavity. The larger end of the pyramidal cell body is next the cavity and a large dendrite which arises from the apex divides into two or more large branches which expand in the fiber layer. The dendrites bear numerous small spines which are knobbed at the end (fig. 26) in the manner characteristic of the epistriatum, inferior lobes, and tectum of Acipenser. These peculiar spines are found nowhere in the brain of Petromyzon except on the epistriatum cells. These cells are so closely similar to the epistriatum cells of Acipenser that it would be impossible to mistake their identity.

The pyramidal body and the dendrites studded with knobbed spines are so characteristic of these neurones that the resemblance to the hippocampal cells in Acipenser, Amia and Rana is at once striking and unequivocal. Examples of these cells in each of the forms named are shown in figures 30 to 34, for comparison with the cells in Lampetra. The bodies of the neurones in Lampetra stand near the ventricle and their dendrites divide into a few relatively straight branches which traverse the thickness of the wall, often reaching the outer surface (figs. 30, 31).

When we look for the boundary between the primordium hippocampi and the epithalamus, the internal structure as seen in horizontal sections seems to furnish the necessary data. First, there is no difference in the internal structure of the whole dorso-median ridge bounded by the foramen and the sulcus limitans hippocampi. Everywhere it is filled by the peculiar type of neurones just described and nowhere is there any change of finer structure which would lead us to say that any two or more parts of it represent different functional centers. However, the moment the sulcus limitans hippocampi is passed in any direction we come upon neurones of types wholly different from those of this ridge. This can not be accidental; it must be the expression of functional differentiation.

The neurones of the so-called striatum have been described and figured in my earlier paper ('02 a, figs 18, 19). They are bipolar or multipolar cells with irregularly curved and branching dendrites free from spines. The neruones of the nucleus habenulae have also been figured ('02 a, fig. 16; this paper, figs. 17 to 20). They are, like those in Acipenser, small cells with short very irregular dendrites often with enlarged tips bearing tufts of small branches. The subhabenular region is broadly continuous with the primordium hippocampi but the boundary line is very distinct in Golgi sections. The type of neurones peculiar to the primordium hippocampi stops abruptly along a line drawn latero-caudad from the sulcus limitans hippocampi (see figures of horizontal sections, 18, 19, 20). At the same time along this same line end abruptly the parallel fibers coursing lengthwise through this ridge. At this line the fibers are cut off in horizontal sections because they are turning up into the nucleus habenulae, as is seen in the most dorsal sections. Compare figures 15, 16 and 19, 20. Behind the line mentioned there is a wholly irregular tangle of nerve fibers and farther back the fibers of the optic tract. Scattered among the tangled fibers are bipolar and multipolar neurones with long sinuous dendrites devoid of spines. The type of structure of the two bodies as a whole is as strikingly different as are the individual neurones found in them.

In transverse sections, owing to the direct contiguity and the oblique overlapping of the primordium hippocampi and the epithalamus, the boundary is of course not so clear, but there is a very abrupt transition from one type of neurones to the other which strongly suggests the distinctness of the two centers.

Tretjakoff ('09) gives a very imperfect description of the 'praethalamus' without figures. He states that its cells are much like those of the thalamus and that the only afferent or efferent tract connected with the praethalamus is constituted by the fibers from the parapineal organ. These reach the praethalamus after crossing in the habenular commissure. The praethalamus serves as a rudimentary perception center for the parapineal eye. "Eine andere Bedeutung des Präthalamus lässt sich bei Ammocoetes kaum vermuten, da ungeachtet der grossen Zahl der Zellen der Präthalamus von Ammocoetes, nach meinen Untersuchungen, keine eigenen aus- oder zu führenden Bahnen hat." The author was evidently impressed with the inadequacy of the parapineal tract to account for so large a center with a great number of cells. This impresses us much more when we remember that the parapineal organ and tract exist only on the left side. According to Tretjakoff's description the fibers cross in the commissure to enter the (right) praethalamus. Hence the left 'praethalamus' is wholly devoid of afferent fibers according to this author, and we are led to suppose that a large center, rich in highly developed cells exists in Ammocoetes totally without function. The writer has found no evidence in his preparations that the parapineal tract enters the 'praethalamus'.

Schilling ('07) is inclined to the opinion that the 'praethalamus' belongs to the telencephalon (p. 431; Herrick cites him erroneously on p. 473) and points out that it is separated from the nucleus habenulae by a deep ventricular sulcus. He did not distinguish the characteristic cells of the 'praethalamus,' but describes the passage through it of the bundles of the taenia thalami which give off collaterals to it.

None of the authors who have studied the petromyzont brain have used methods adequate to the differentiation of the types of cells characteristic of the so-called praethalamus and the parts adjacent to it. No method is so well adapted to this purpose as the Golgi method and the description of this region given by the writer in 1902 stands as the most complete heretofore given. That description gave, however, a very incomplete account of the facts shown in my preparations and the deficiency is to some extent made up in the figures accompanying this paper. The 'praethalamus' of authors is sharply distinguished from the epithalamus and thalamus not only by the ventricular sulci but by the well marked characteristics of its cells and by the course of fibers which traverse it.

Between this body and the roof of the hemisphere there is in the same way a clear difference in the type of cells. This has been sufficiently illustrated earlier ('02 a) and there is no dispute among authors upon this point.

The fiber tracts related to the primordium hippocampi

These fiber tracts have been very imperfectly understood. Both Schilling ('07, p. 432) and Tretjakoff ('09, p. 731) state that no tracts connected with this nucleus were seen.

The stria medullaris is perhaps less complex in petromyzonts than in the higher fishes, but at least four bundles related to the telencephalon are present. (Fibers connecting the epithalamus with other parts of the diencephalon will not be considered here). One of these bundles comes from the medial olfactory nucleus, enters the primordium hippocampi near its rostral end above the foramen and seems to pass over the dorsal ventricular surface of this body to enter the nucleus habenulae (figs. 14 to 19, 25, 26, 35). It is by no means certain that this is a continuous tract.

A tract from the medial olfactory nucleus over the foramen interventriculare to the nucleus habenulae is not known in other vertebrates. The fibers passing up from the medial olfactory nucleus as far as the primordium hippocampi occupy the same place as the tractus olfacto-corticalis septi in selachians and amphibians. If these fibers end in the primordium hippocampi, then what appears to be the continuation of them to the nucleus habenulae must be classed as cortico-habenular fibers. A second bundle comes from the lateral and caudal walls of the hemisphere, passes up behind the foramen through the inner and lower part of the primordium hippocampi to join with the first as it enters the nucleus habenulae (figs. 17, 18, 19). This is the tractus olfacto-habenularis of authors. A third bundle comes from the preoptic region and joins the first two (fig. 35). These three bundles unite into a large compact bundle which traverses the eminentia thalami at the bottom of the prehabenular recess and enters the nucleus habenulae (figs. 19, 26, 27). The fourth component of the stria medullaris is very diffuse and comes from the whole thickness of the primordium hippocampi. Axones arising from the cells of the primordium are seen in many cases passing at first peripherally among the dendrites of these cells and then turning to run toward the nucleus habenulae through the substance of the primordium hippocampi. It is these fibers in addition to the first and second bundles above described that give a longitudinal striation to the whole of this body. At the caudal end of the primordium these fibers enter the nucleus habenulae caudal and somewhat dorsal to the compact bundle of the stria medullaris (figs. 16, 17).

The left nucleus habenulae is very small in Lampetra and nearly all the left stria medullaris enters the superior commissure. In the preparations from which figures 14 to 22 were drawn no fibers were seen ending in the left nucleus. The view expressed in the writer's paper on Acipenser ('01, p. 115) that the larger size of the right nucleus is correlated with the ending of a larger portion of the tractus olfacto-habenularis in the right nucleus is strongly supported in Lampetra. The superior commissure presents two main divisions, a more rostral, compact bundle and a more caudal portion made up of several strands (figs. 17, 18).

The rostral bundle is composed of fibers from the first bundle described above and its fibers seem to end in the fiber-mesh of the right nucleus (figs. 18, 19). The corresponding bundle of the right side meets with the left and seems to end with it in the right nucleus.

The strands of the more caudal division of the commissure are made up of fibers of the remaining three bundles intermingled. Many of these fibers end in the fiber mesh of the right nucleus but many others pass through the nucleus without ending. This is especially evident in such horizontal sections as those drawn in figures 17 to 20. At least a part of these fibers clearly appear to be those which arise in the primordium hippocampi. If so, it is probable that these fibers end in the corresponding body on the other side and are homologous with the posterior pallial commissure which is prominent in selachins, ganoids, teleosts and amphibians.

The fibers in the caudal division of the commissure which end in the right nucleus may include those parts of the tractus olfacto-habenularis which in other fishes arise in the lateral olfactory nucleus and in the nucleus praeopticus. These may also include fibers from the primordium hippocampi which would belong to the tractus cortico-habenularis.

It seems reasonably certain from the study of these Golgi sections that the stria medullaris contains the equivalent of the tractus olfacto-habenularis lateralis and posterior, and the commissura palli posterior as described in the selachian brain. Nearly all of these fibers from the left side cross in the superior commissure.

The writer has described ('02 a, pp. 38, 40) two afferent tracts ending in the primordium hippocampi; one ascending from the hypothalamus and one coming from the formatio bulbaris. The former has since been called tractus pallii in all fishes and is regarded as the ascending gustatory tract entering the telencephalon for the sake of correlation of gustatory with olfactory impulses. This tract decussates in the post-optic commissure and ascends over the internal face of the tractus opticus to enter the primordium hippocampi from below and behind.

The fibers which enter the rostral end of the primordium hippocampi from the formatio bulbaris are of course part of the olfactory tract. In the previous description ('02a, p. 40) it was stated that these come chiefly from the opposite side, crossing in the olfactory decussation. The contribution of the commissure to the fiber bundle entering the primordium hippocampi is illustrated in figure 24. Further study, both of the preparations used at that time and of new preparations, has shown, however, that a much larger number of direct fibers are present than was previously thought. These fibers are soon mingled with the fibers of the first and second bundles of the stria medullaris mentioned above and can not be distinguished with certainty. It is clear, however, that many fibers which enter from in front break up into end branches in the primordium hippocampi. Since we know of no other animals in which olfactory tract fibers run without relay to the nucleus habenulae, the presumption is strong that these olfactory tract fibers end in the primordium hippocampi. This is the more probable in view of the fact that in selachians corresponding fibers are present which end in the primordium hippocampi in part on the same side and in part after crossing in a commissure situated above the neuroporic recess.

Schilling and Tretjakoff both state that the fibers of the tractus olfacto-habenularis give collaterals to the 'praethalamus.' Such endings would represent a rudimentary tractus olfacto-corticalis. On comparative grounds the presumption is strong that those fibers mentioned above which run up form the medial olfactory nucleus rostral to the interventricular foramen to enter the primordium hippocampi must constitute a tractus olfacto-corticalis.

The efferent tract from the primordium hippocampi has thus far been very imperfectly understood. The writer described fibers descending to the 'striatum' and these have been confirmed by Tretjakoff. These fibers are fine and do not appear in sufficient numbers in my preparations to warrant the conclusion that they represent the chief or only efferent path of this highly differentiated nucleus. Neither is it clear that they end in the 'striatum'. It is possible that they pass through the 'striatum' but are not impregnated beyond. Other fibers may descend in the tractus pallii, as this tract in selachians and ganoids contains descending fibers. The pathway by which the fornix columns run in all higher classes is occupied by a broad bundle of fibers connecting the medial olfactory nucleus with the primordium hippocampi (see above). If any fibers descend through this to reach the hypothalamus, they would represent the fornix columns. We must await further investigations upon these points.

We may now summarize the evidence for the interpretation of this 'praethalamus' or 'dorso-median ridge,' which has been assumed in using the name primordium hippocampi.

(a) Its caudal end is just in front of the eminentia thalami to which the velum transversum is attached. It is therefore wholly within the telencephalon.

(b) It contains highly developed and specialized cells of a type which is characteristic of the primordium hippocampi in selachians ganoids, teleosts and amphibians.

(c) It is bounded by a ventricular sulcus which agrees closely in position with the sulcus limitans hippocampi of selachians, ganoids and teleosts.

(d) Along the line of this sulcus there is a sudden change from the characteristic cells to cells of very different form in the thalamus, epithalamus and hemisphere. This abrupt change of structure is comparable to the zona limitans of fishes and amphibians.

(e) It is traversed by a part of the tractus olfacto-habenularis as in ganoids and teleosts.

(f) It has true commissural fibers passing through the superior commissure as in fishes and amphibians(commissura pallii posterior).

(g) It receives from in front fibers of the olfactory tract, direct and crossed, comparable to those in selachians and in part to those of ganoids and amphibians.

(h) It receives a tractus pallii ascending from the hypothalamus as in all fishes. The center is therefore to be regarded as an olfacto-gustatory correlation center.

(i) It appears probable that there is a tertiary olfactory tract ending in this body (tractus olfacto-corticalis).

Admitting such uncertainty as exists in the present state of our knowledge regarding the posterior pallial commissure and the tertiary olfactory connections, we have here a body of evidence which leaves no reasonable ground for doubt that the body in question is the homologue of the primordium hippocampi as described in fishes and amphibians.

There is no ground whatever for Professor Herrick's assumption ('10, p. 473) that the greater part of this body belongs to the epithalamus, while a smaller rostral portion represents the primordium hippocampi.

The fact of greatest interest regarding this body is, perhaps, that the primordium of the visceral cortical complex of higher animals (hippocampus, fornix, and related structures) remains in cyclostomes in the telencephalon medium. As has been pointed out in previous papers ('10 c, '11 a) the hemisphere evagination in vertebrates involves at first only the primary olfactory centers and it is only in later stages of phylogeny that the cortical substance is evaginated.

Anterior pallial commissure

This has been known as the dorsal olfactory decussation, the dorsal part of the anterior commissure, and by other names. Schilling speaks of this as the anterior commissure and makes only minor mention of the true anterior commissure. Schilling recognizes in this dorsal commissure fibers connecting the formatio bulbaris of the two sides and fibers connecting each formatio bulbaris with the opposite lobus olfactorius. The writer has described fibers connecting the lobus of one side with the opposite primordium hippocampi. In addition, there are to be mentioned fibers which come to the commissure from the area of union of the caudal wall of the hemisphere with the telencephalon medium (fig. 22). A certain determination of the nature of these fibers depends on the further study of the region from which they arise. They may correspond to the fibers in selachians to which the writer has given the name corpus callosum. The matter of greatest moment in this connection is the existence in the lowest order of vertebrates of a commissure located in the lamina supraneuroporica. The writer has shown elsewhere that the corresponding commissure in selachians is large and important, and must reiterate the view that this dorsal or supraneuroporic commissure is primitive and fundamental in vertebrates. A further examination of the pallial commissures in reptiles and mammals with reference to this is in progress (see '10 c).

Anterior commissure

This commissure is rather small in cyclostomes. It lies in the lamina terminalis in front of the preoptic recess as in all vertebrates. Its constitution is not at all clearly known. Its fibers probably come from the basal (lateral) olfactory area and the so-called 'striatum.'

The 'striatum'

The region to which the name striatum has been applied includes the supraoptic portion of the telencephalon medium and an adjacent part of the hemisphere (figs. 5, 6, 22). It is clear that this corresponds roughly to the striatum of other fishes. The writer has shown ('02 a, p. 41) that the descending fibers from the 'striatum' go to the thalamus and not to the hypothalamus. From this region fibers pass through the anterior pallial commissure, as noted above. These facts suggest that the striatal region corresponds to or contains the equivalent of the somatic area of selachians. Any decision on this point must await further study.

Mode of evagination of hemispheres. Zona limitans

The cyclostomes taken in comparison with higher forms give clear evidence of the principle announced by the writer ('10 c, '11 a) that the hemispheres are lateral evaginations of the telencephalon which have gradually involved the formatio bulbaris, the lobus olfactorius and the pallium in the order named. The comparison of the cyclostomes with selachians and amphibians with reference to the mode by which the more fully evaginated brains have come to have their present form, is very important for the interpretation of these higher brains.

If attention be given to the models shown in figures 5, 6 and 7, it will be seen that the further evagination of the hemisphere involves the turning out of the primordium hippocampi and the striatal area through the foramen into the caudal portion of the hemisphere. Somewhat more than the rostral half of the hemisphere is occupied by formatio bulbaris. The further evagination would enlarge the caudal part of the hemisphere until it became larger than the rostral part. At the same time the hemisphere is drawn forward by the elongation of the rostrum, the olfactory bulb becomes separated form the caudal part of the hemisphere and a longer or shorter tractus olfactorius or olfactory peduncle is established.

The relations of the primordium hippocampi to the roof of the enlarging hemisphere are especially interesting. We have pointed out that there is an abrupt change of structure between the primordium and the roof of the hemisphere which may be called a zona limitans. As the primordium hippocampi pushes out into the roof of the hemisphere this zona limitans would be placed successively farther and farther laterad in this roof. In selachians ('11 a) this zona limitans runs from the olfactory peduncle backward along the dorso-lateral wall of the lateral ventricle. For a long time in the phylogeny there remains a portion of the primordium hippocampi in the telencephalon medium extending along the dorsal border of this region to the point of attachement of the velum transversum to the eminentia thalami. In this position, where the whole of the primordium is located in cyclostomes, there exists in Chimaera ('10 d) and selachians ('11 a) a slender ridge of gray matter accompanied by the fibers of the posterior pallial commissure. In ganoids and teleosts ('11 b), where the evagination of the hemispheres has been arrested, the primordium has retained in the caudal part of the telencephalon essentially the relation which it holds in cyclostomes and has taken part in the eversion of the forebrain. In amphibians the evagination has gone farther than in selachians, the primordium hippocampi occupying the roof portion of the hemisphere and the zona limitans being carried farther down in the lateral wall. There is still left in the telencephalon medium, however, a small part of the primordium hippocampi corresponding to that in selachians, although shorter (Johnston, '06). Herrick ('10, p. 476) has endeavored to show that this structure belongs to the eminentia thalami. It is of course directly adjacent to the eminentia thalami and Professsor Herrick has apparently thought that more was to be included in the unevaginated primordium hippocampi than the writer intended. This structure is the continuation of the fimbria-border of the hemisphere into the dorsal border of the telencephalon medium until it meets the eminentia thalami. This small remnant of the primordium is seen in a model of the forebrain of Necturus standing vertically rostral to the eminentia thalami and separated from the latter by a groove. Figure 36 will show more clearly the relations of this structure. Deep fibers related to the cells of this unevaginated body enter the hippocampal commissure as already described.

As the evagination proceeds the caudal part of the hemisphere soon extends rostrad beyond the level of the interventricular foramen and the lamina terminalis. This is already true in selachians and in amphibians and reptiles the hemisphere protrudes far rostrally (fig. 37). In selachians, it has already been pointed out that the sulcus limitans hippocampi continues rostrally beyond the laterally placed olfactory peduncle and encircles the rostral wall of the hemisphere to end at the neuroporic recess (figs. 38, 39). This portion of the sulcus was called the medial sulcus limitans hippocampi. As compared with cyclostomes, the condition in selachians has resulted from the continued evagination which has carried the olfactory bulbs far laterad and has brought the greater part of the primordium hippocampi into the roof of the hemisphere. The region of the medial olfactory nucleus has bulged forward beyond the lamina terminalis, and the sulcus which separates the primordium from the medial olfactory nucleus in front of the interventricular foramen in Petromyzon comes in selachians to lie in the medio-rostral wall of the hemisphere. In both classes it runs from the neuroporic recess into the lateral ventricle and marks the line of separation of the same structures. It is properly called the sulcus (or zona) limitans medialis. In amphibians the conditions are essentially the same (fig. 37).

Professor Herrick's view as to the evagination of the hemispheres and the relation of his four columns to them is summarized in the following paragraph ('10, p. 477):

The roof plate and floor plate converge into the lamina terminalis, where of course they end. The four massive columns on each side converge into the interventricular foramen, and in larvae with wide foramina and adult urodeles they may be followed through the foramina into the evaginated hemispheres. Bearing in mind the fact that during development the roof plate and floor plate retain permanently their primitive attachments to the lamina terminalis, and that it is only the massive lateral columns which are evaginated into the hemispheres, it clearly follows that these columns of the diencephalon are continued into the hemispheres in the form shown by the accompanying diagram (fig. 84), the zona limitans lateralis representing the locus of the sulcus medius and the zona limiants medialis the line of union of the dorsal and ventral columns in the lateral evaginations rostral to the fusion of the roof plate and floor plate in the lamina terminalis.

It has been noted elsewhere ('11 b, p. 540) that the assignment of the lamina terminalis in this paragraph to the floor plate was an error of inadvertance. It has been shown also ('11 b, pp. 534, 535 and in this paper) that the sulcus diencephalicus medius has no relation with the interventricular foramen or the zona limitans lateralis either in amphibians, fishes or cyclostomes.

The conception of the folding over of the lateral walls of the diencephalon illustrated in Professor Herrick's figures 83 and 84, such that the dorsal and ventral borders fuse in the medial zonae limitantes to form two tubes extending forward (the hemispheres), seems to the writer to be without basis in fact.

In figure 72 Herrick ('10) has given a diagram of the forebrain in a hypothetical vertebrate ancestor. In this the representation of the sulcus dorsalis is subject to the criticisms made above (p. 352). No ground is given for the assumption that the retinal area does not involve the dorsal border of the brain, except the erroneous supposition that there is no di-telencephalic fissure in cyclostomes. The sulcus medius is represented as a horizontal sulcus extending caudad from the site of the interventricular foramen, whereas in all lower vertebrates it runs nearly vertically up into the dorsal sac. The sulcus ventralis should lead to the site of the interventricular foramen as it does in cyclostomes, selachians and amphibians. The terminal ridge is represented as an independent thickening rostral to the chiasma ridge, whereas the terminal ridge in all vertebrate embryos itself becomes the bed for the optic chiasma (Johnston '09).

SUMMARY

The evagination of the hemispheres is at a low stage in petromyzonts. In Ichthyomyzon and Lampetra the formatio bulbaris is not all evaginated. The fact that a part of the wall of the third (median) ventricle in these forms is made up of formatio bulbaris ought to be convincing evidence, if any further evidence is needed, that a part of the third ventricle belongs to the telencephalon. A large part of the secondary olfactory centers is evaginated, but a relatively much larger portion of these centers remains in the telencephalon medium than in the case of selachians. The primordium hippocampi remains wholly in the telencephalon medium. The exact relations of the somatic area await further study.

The di-telencephalic boundary is marked by the attachement of the velum transversum to the eminentia thalami as in selachians ganoids and amphibians. The eminentia thalami is a well-marked ridge just rostral to the nucleus habenulae, bounded by the sulcus medius and by the sulcus limitans hippocampi, and traversed by the stria medullaris.

The telencephalon medium, which is relatively larger than in higher vertebrates, stands as a wedge-shaped mass between the hemispheres. Its massive nervous walls are connected with one another by the lamina terminalis, lamina supraneuroporica and tela chorioidea. The membranous roof plate (His) in the telencephalon of petromyzonts connects the two simple unevaginated lateral walls of the neural tube as in any other segment. The hemispheres are evaginations of restricted areas of these lateral walls. The foramen is roofed over by the massive primordium hippocampi and there is no extension of the tela chorioidea into the roof of the hemispheres.

The primordium hippocampi is represented by the 'praethalamus' of previous authors (the epistriatum of my earlier paper, '02 a). It is a large body having a highly developed and characteristic finer structure which already presents most of the relations of the primordium hippocampi of higher forms (see text). The two primordia converge forward to form a bed for the anterior pallial commissure in the lamina supraneuroporica. The primordium is separated from the epithalamus, thalamus, hemisphere and the medial olfactory nucleus by a sulcus limitans hippocampi. Along the line of this sulcus is a sudden change of structure which may be compared with the zona limitans of fishes and amphibians.

In vertebrates above cyclostomes the primordium hippocampi is gradually evaginated into the hemisphere, the process being complete in the reptiles. It is only as the hippocampus is carried into the medial border of the hemisphere that the tela chorioidea comes to form the roof of the interventricular foramen and to extend into the roof of the hemisphere.

The expansion of the hemisphere carries both the primordium hippocampi and the medial olfactory nucleus rostrad beyond the lamina terminalis where they form the medial hemisphere wall. The sulcus limitans hippocampi which separates these two nuclei in the telencephalon medium in petromyzonts becomes the sulcus limitans medialis hippocampi in the hemisphere of selachians, amphibians and reptiles.

The relation of the primordium hippocampi to the lamina supraneuroporica in which the anterior pallial commissure lies is fundamental in vertebrates and must be taken into account in seeking the interpretation of higher brains.

The reader is referred to the extended discussion of forebrain morphology contained in the writer's paper on selachians ('11 a, p. 37, especially pp. 47–52). The features of the evolution of the forebrain upon which the cyclostome brain throws light especially are briefly the following.

(a) The hypertrophy, rising dorsad and eversion of the olfacto-gustatory correlation center. The same thing occurs in the visceral receptive column in the medulla oblongata of many fishes and the nucleus habenulae commonly.

(b) The presence originally of the whole olfactory central apparatus in the wall of the medial ventricle. Hemispheres are formed by the evagination first of formatio bulbaris, then secondary olfactory centers, and last the visceral and somatic correlating centers which furnish the materials for the development of the cortical structures.

(c) From a condition like that of cyclostomes the selachian telencephalon has been derived by the evagination chiefly of the medial olfactory nucleus and the primordium hippocampi. In (Chimaera), ganoids and teleosts the evagination has been arrested and the eversion of the primordium hippocampi seen in cyclostomes is greatly increased. The formatio bulbaris actually bounds the median ventricle near the foramen and the apparent folding out of of the striatal or somatic area in cyclostomes is absent in ganoids and teleosts (see '11 b).

(d) The primitive relations of the hippocampal primordium and of its commissure in the lamina supraneuroporica are splendidly clear and instructive in cyclostomes. The selachians present essentially the same relations in evaginated brains. In ganoids and teleosts ('11 b) the extreme eversion has abolished the primitive supraneuroporic commissure, except in a few forms. In the amphibians the commissures are similar to those of ganoids. In reptiles and mammals the cyclostome and selachian condition reappears.

(e) The view of the general morphology of the telencephalon previously expressed ('10 c, '11 a, '11 b) receives the strongest support from these most primitive brains. The functional columns of the brain described by the writer ('02 b) extend forward to the telencephalon. The ventral columns are represented only by correlating tissue adjacent to the terminal ridge or optic chiasma. The dorsal columns constitute the overwhelming part of the telencephalon, are greatly hypertrophied and become flexed in consequence. The dorsal and ventral columns meet in the preoptic recess where the sulcus limitans of His ends. The telencephalon in petromyzonts is not elongated parallel with the long axis of the fish, but owing to its flexure, is longer dorso-ventrally.

The olfactory nerve and sac on the one hand and the formatio bulbaris on the other are closely related to the neuroporic recess and illustrate clearly the principle ('11 a, p. 39) that the neuroporic recess owes its existence to the attachment of the olfactory nerve to the dorsal lip of the neural tube at this point. The evagination of hemispheres began here close to the dorsal border at some distance from the anterior end of the brain tube. This point lies at about the middle or height of the forebrain flexure.

Caudal to this middle point of the forebrain the visceral receptive column forms the olfacto-gustatory correlation center or primordium hippocampi. From the study of other fishes ('11 a and '11 b) I have shown reason to think that the somatic correlation center, or primordium of the somatic cortex has been formed by eversion and migration of neuroblasts from the dorsal border of the telencephalon in this caudal part.

The cephalic part of the forebrain comes to be ventrally placed owing to the flexure, and is constituted chiefly or wholly of the medial and lateral secondary olfactory centers and preoptic nucleus. The formatio bulbaris arises from tissue nearest the olfactory nerve root at the middle part of the dorsal column and in higher forms is carried out upon a peduncle. In some petromyzonts it actually retains its relation to the median ventricle.

An understanding of the fundamental morphological and functional relations in the forebrain must be built upon the central fact of the forebrain flexure. The conception of a curved axis of the telencephalon ending at the preoptic recess, and of the olfactory nerve entering the dorsal border at the height of the curve must never be lost sight of. These are fundamental facts, not hypotheses, and all forebrain morphology becomes confused unless these facts are held firmly in mind.

In forms above the cyclostomes the rostrum elongates, the olfactory sac shifts forward, the bulbar formation follows it and becomes pedunculated. At the same time the evagination of hemispheres progresses rapidly and the hemispheres elongate. Now the cephalic and caudal portions of the visceral receptive column are sharply flexed on one another in the form of a letter *U* (figs. 40, 41). The olfactory tract enters the curve of the *U*. The cephalic limb, containing the secondary olfactory centers is ventrally placed and ends at the preoptic recess. The caudal limb (primordium hippocampi) is dorsally placed and ends at the eminentia thalami. The two limbs are separated by the interventricular foramen and by the medial and lateral zona limitans as already explained.

Owing to the eversion and migration of the neuroblasts of the somatic correlating center down upon the lateral surface of the telencephalon this primordium of the general cortex comes to lie between the limbs of the *U*. In this position it enters into relations with the lateral olfactory nucleus which lead to the formation of the pyriform lobe as an olfacto-somatic correlation center (cortex). Within the somatic primordium there develop also complex correlation systems between the cutaneous, musculo-sensory, auditory and visual fiber tracts which probably reach this center at an early stage in the phylogeny (see '10 c, and '11 a). This complex correlating organ is the general cortex. As it develops it pushes in between the primordium hippocampi and the pyriform lobe, assuming the characteristic postion of the general cortex in mammals. The expansion of the general cortex pushes the hippocampal formation to the medio-dorsal border of the hemisphere and the pyriform lobe down to the ventral surface.

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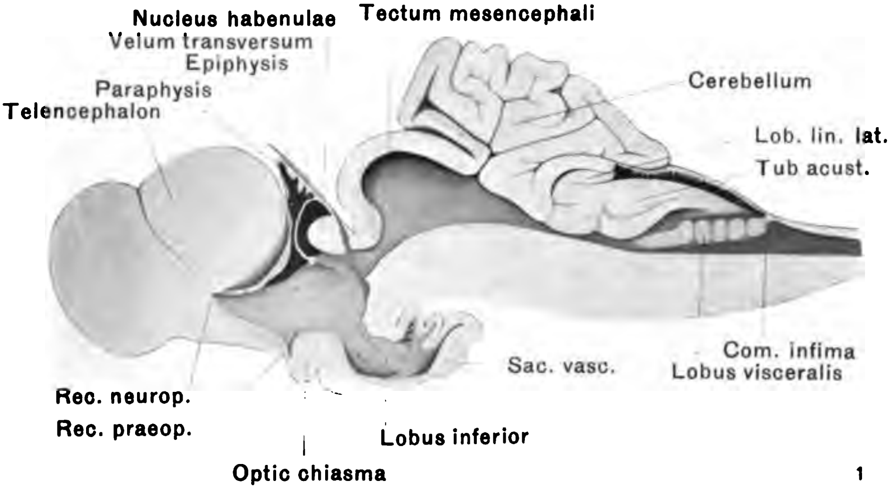
REFERENCE LETTERS

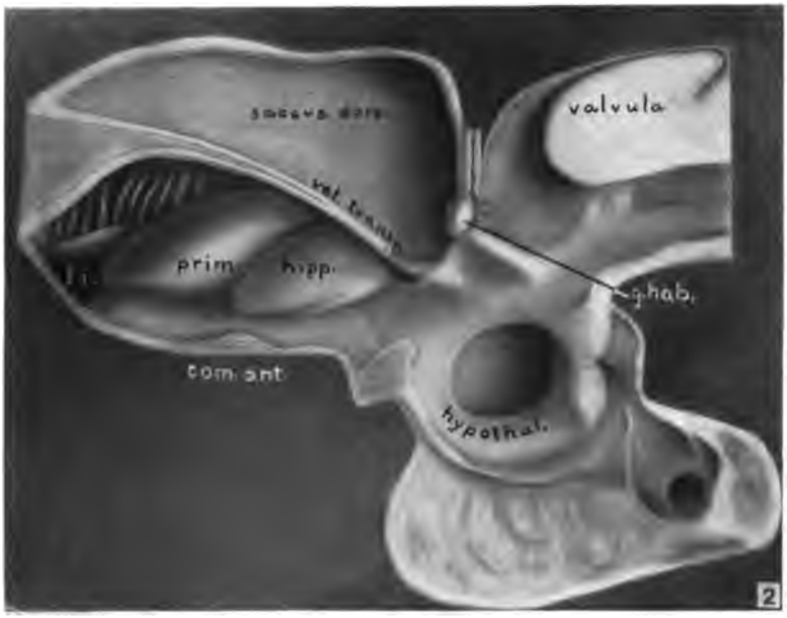
b.o., bulbus olfactorius, formatio bulbaris.
c. a., commissura anterior
ch., chiasma opticum.
c.hipp., commissura hippocampi.
c.p., commissura posterior.
c.p.a., commissura pallii anterior.
c.p.p., commissura pallii posterior.
c.sup., commissura superior (Osborn).
dienc., diencephalon.
di-tel., di-telencephalic boundary.
d.p., decussatio postoptica.
e., epiphysis.
em.th., eminentia thalami.
f.i., foramen interventriculare.
f.olf., fila olfactoria.
f.p.p., fibers from the parapineal body.
g., olfactory glomeruli.
hem., hemisphere.
hy., hypophysis.
hyp., hypothalamus.
l.o., lobus olfactorius (secondary centers).
l.s., lamina supraneuroporica.
l.t., lamina terminalis.
l.v., lateral ventricle.
m., caudal margin of lamina supraneuroporica.
mesenc., mesencephalon.
n.h., nucleus habenulae.
n.olf., nervus olfactorius.
nuc.olf.med., nucleus olfactorius medialis.
par., paraphysis.
p.c.b., precommissural body.
p.h., primordium hippocampi.
p.p., parapineal body.
pt., pretectal region.
rec.ph., recessus praehabenularis.
r.m., recessus mammillaris.
r. n.,recessus neuroporicus.
r.p., recessus praeopticus.
r.po., recessus postopticus.
s.d., saccus dorsalis.
s.l., sulcus limitans hippocampi.
s.l.H., sulcus limitans of His.
s.m., stria medullaris.
s. m. 1, first bundle of stria medullaris
s.med., sulcus diencephalicus medius of Herrick.
s.hy., sulcus hypothalamicus.
sub.hab., subhabenular region.
t.c., tela chorioidea.
tel.m., telencephalon medium.
t.f., taenia fornicis.
thal., thalamus.
t.p., tuberculum posterius.
tr.c-h., tractus cortico-habenularis.
tr.h-p., tractus habenulo-peduncularis.
tr.o-h., tractus olfacto-habenularis.
tr.op., tractus opticus.
tr. pall.,tractus pallii.
v. tr., velum transversum.

Fig. 1 The medial surface of the right half of the brain of Mustelus to show the position of the velum transversum in selachians. It is attached to a slight eminentia thalami which is not lettered.

Fig. 2 Medial view of the right half of the telencephalon and diencephalon of an adult Acipenser. The olfactory bulb is not drawn. The velum transversum is a very deep fold of the tela chorioidea which is nearly horizontal in position. It is attached to the eminentia thalami as in selachinas, amphibians and others.


Nucleus habenulae
Tectum mesencephali
Velum transversum
Epiphysis
Paraphysis
Telencephalon
Cerebellum
Lob. lin. lat.
Tub acust.
Com. infima
Lobus visceralis
Sac. vasc.
Rec. neurop.
Rec. praeop.
Lobus inferior
Optic chiasma

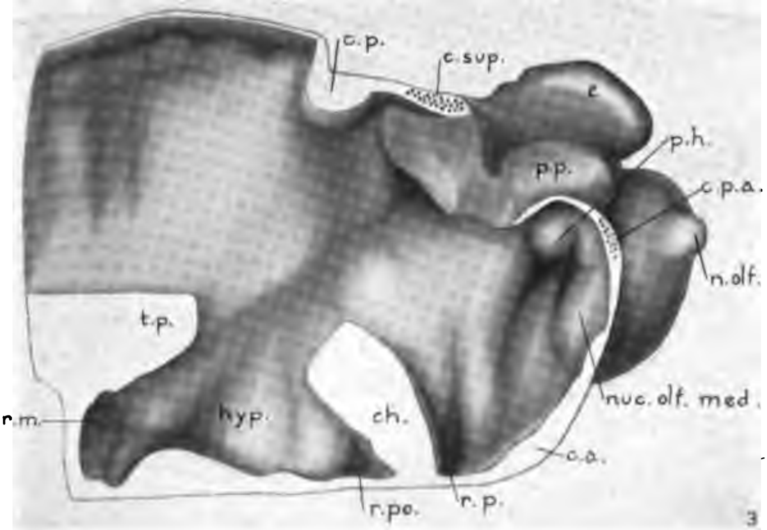
1


saccus dors.
valvula
vel. transv.
l. t.
prim. hipp.
g. hab.
com. ant
hypothal.

2

Fig. 3 Petromyzon dorsatus, ammocoetes. Medial view of a model of the left half of the forebrain.

Fig. 4 Lateral view of the model shown in figure 3. The hemisphere is divided by a vertical sulcus into a rostral portion containing the bulbar formation and a caudal portion containing the secondary olfactory centers. About opposite the caudal pole of the hemisphere appears a vertical sulcus in the wall of the brain stem. This corresponds in position to the di-telencephalic boundary line as determined by other data and is to be regarded as a di-telencephalic sulcus or fissure.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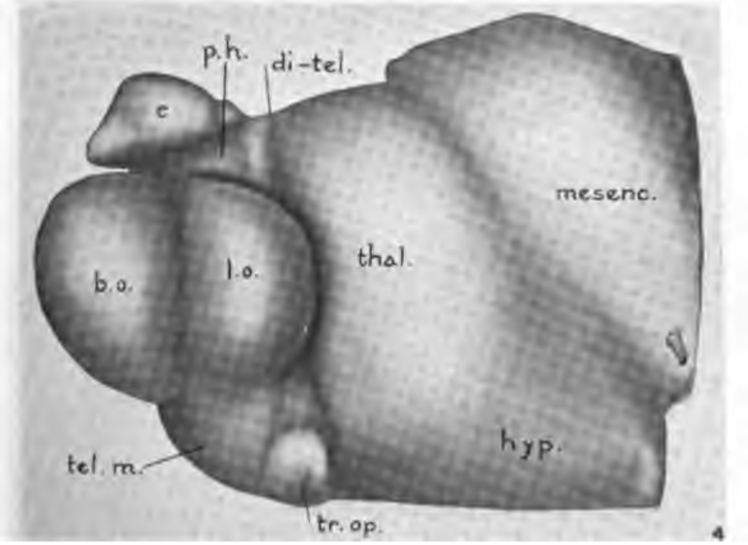
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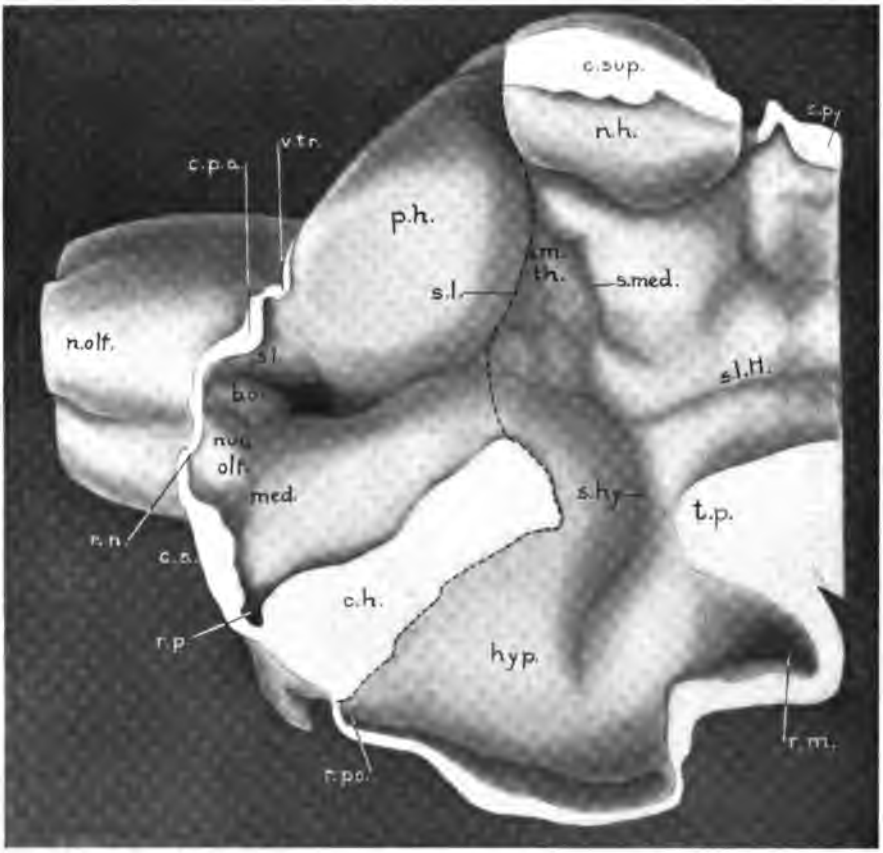


Fig. 5 Lampetra wilderi. Medial view of the right half of a model of the telencephalon and diencephalon. × 50.

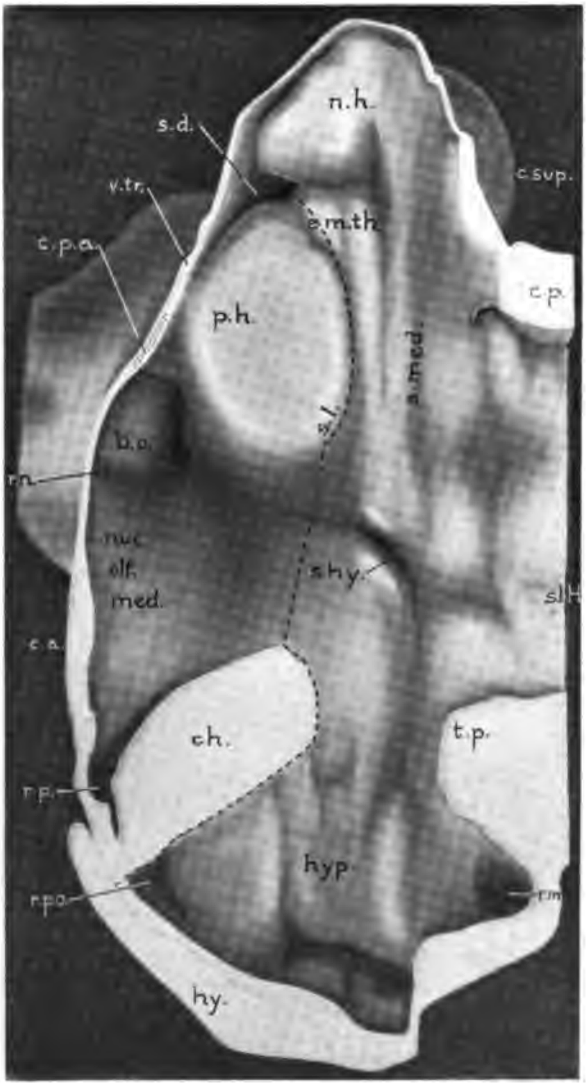


Fig. 6 Ichthyomyzon concolor. Medial view of the right half of a model of the telencephalon and diencephalon. × 66. The slender groove rostral to the sulcus medius is apparently due to one or two sections being less stretched in mounting than the adjacent ones.

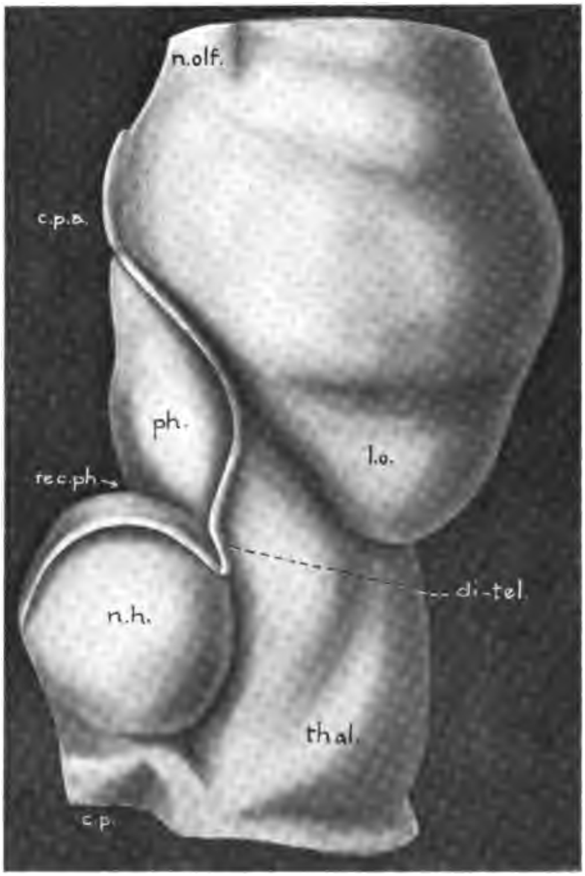


Fig. 7 Lampetra. Dorsal view of the model shown in figure 5. This is presented chiefly to illustrate the stem-hemisphere fissure and the wedge-shaped telencephalon medium. The attached border of the tela chorioidea is drawn. Its caudo-lateral angle is the upper end of the recessus praehabenularis. In Petromyzon dorsatus the eminentia thalami comes up nearly to this point and the velum transversum is attached to it. The broken line running caudo-laterad from this point corresponds to the plane of sudden transition in internal structure from the primordium hippocampi to the pretectal and subhabenular region.

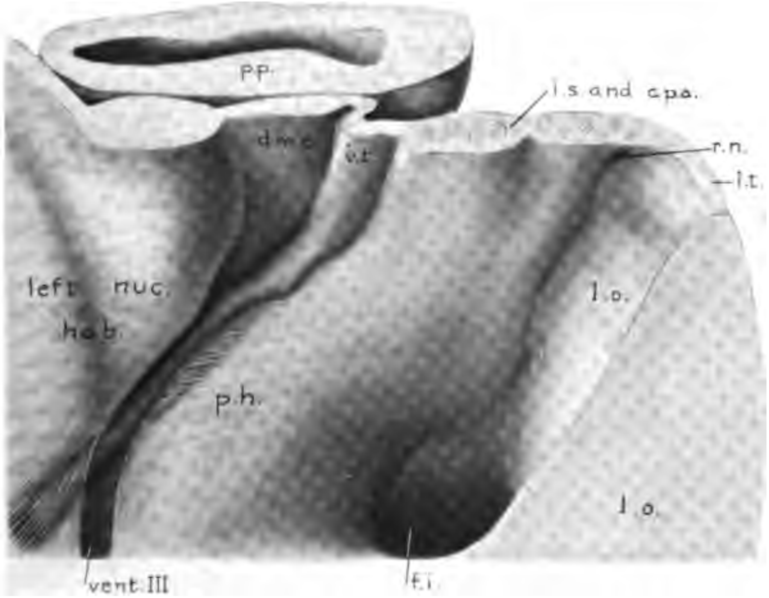


Fig. 8 Petromyzon dorsatus, ammocoetes. Model of the dorsal portion of the left half of the forebrain, drawn as seen from the medio-ventro-caudal direction. The model was carefully constructed on a large scale in order to determine whether the velum transversum really formed a continuous fold from the median line to the lateral attachment of the tela (i. e., the taenia). This drawing should be compared with figures 23 to 27. *d.m.c.*, dorsal sac.

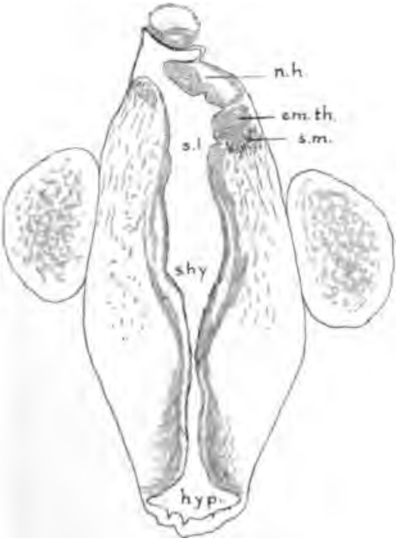
Fig. 9 Ichthyomyzon concolor. A transverse section through the nucleus habenulae and eminentia thalami of the right side. The section cuts the eminentia thalami at its most prominent part, where it is bounded by the sulcus limitans hippocampi below and the sulcus subhabenularis above, as seen in figure 6.

Fig. 10 Petromyzon dorsatus, adult. Transverse section through the interventricular foramen. The primordium hippocampi is not so large as in Lampetra. At the lip of the foramen it is continuous with the wall of the hemisphere.

Fig. 11 Same series as figure 10, section through the eminentia thalami.

Fig. 12 Entosphenus, ammocoetes of 35 mm. Transverse section through rostral part of foramen interventriculare. The section is quite oblique so that on the right side it passes considerably rostral to the foramen. The primordium hippocampi is small but occupies the same position as in other forms. The section falls at the junction of the parapineal body and the left nucleus habenulae.

Fig. 13 Same series as figure 12, section through the eminentia thalami and the habenular nuclei. Note the great size of the right nucleus habenulae. On the right side the primordium hippocampi is cut.



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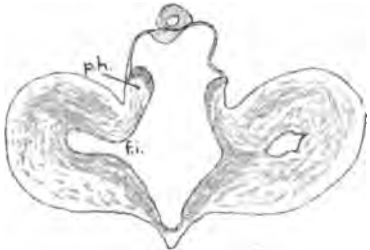

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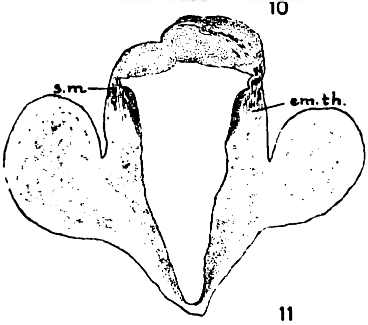

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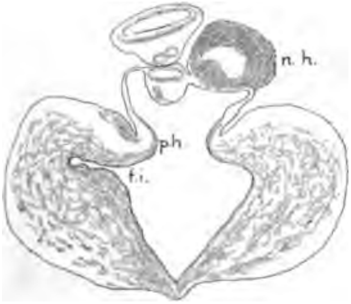

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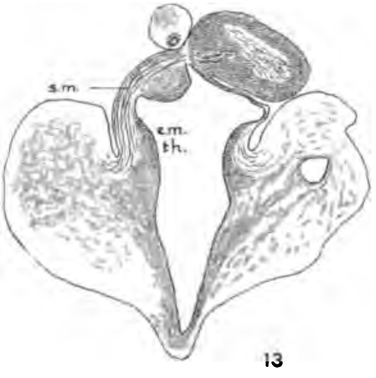

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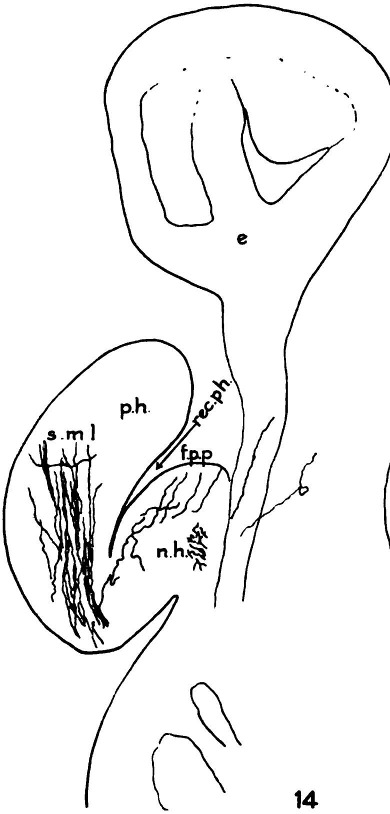
Figs. 14–22 Lampetra. Nine sections of the habenular and hippocampal region drawn from a series of horizontal sections prepared by the Golgi method. The figures were drawn at a magnification of 200 diameters and are reduced to one-third. Figure 22 is at a somewhat lower magnification.

Fig. 14 The first section through the dorsal part of the left primordium hippocampi. The section shows the epiphysis and its stalk. The four fibers in the left nucleus habenulae come from the parapineal body. The bundle *s.m.1* is the first part of the stria medullaris of which it is uncertain whether it is a tractus olfacto-habenularis or tractus cortico-habenularis.

Fig. 15 The second section of the same series. The first bundle of the stria medullaris is passing through the left nucleus habenulae.



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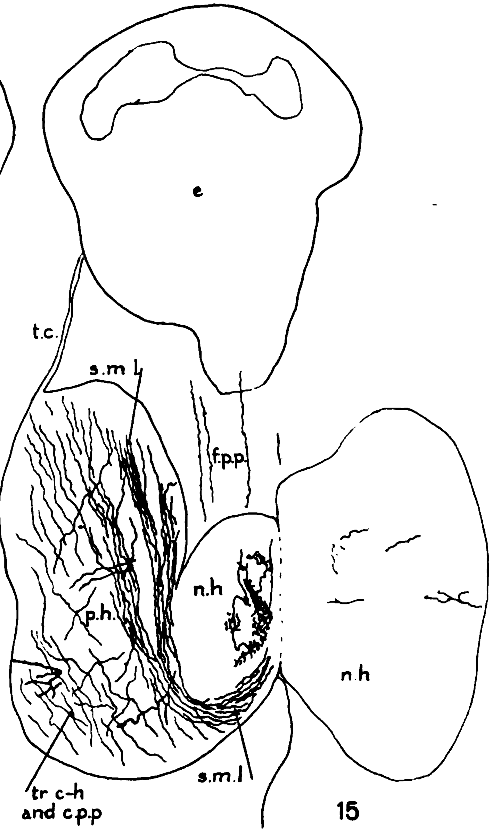

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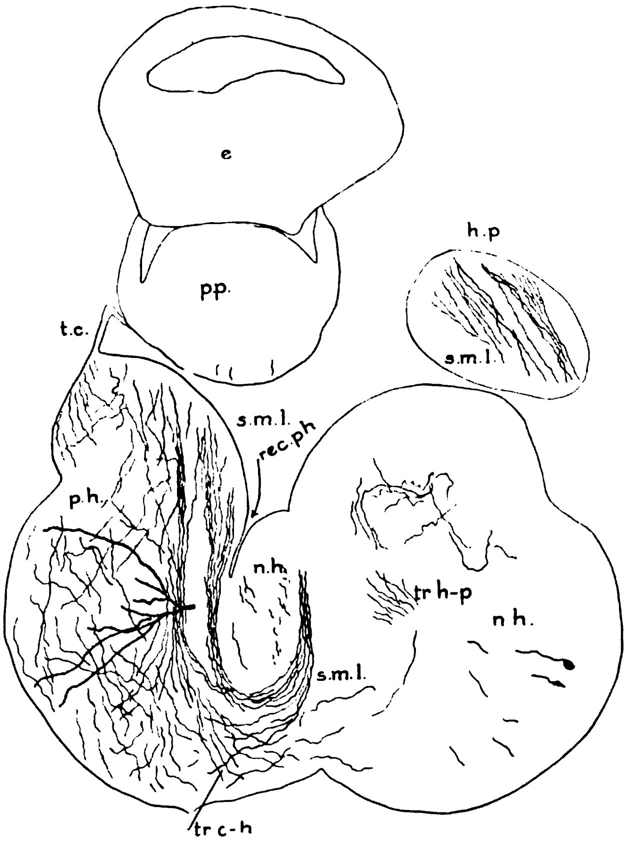


Fig. 16 The third section of the same series. Note the extreme difference in size between the two nuclei habenulae and the passage of the first stria medullaris fibers as a compact bundle over to the right nucleus. The prehabenular recess is narrow in Lampetra and the eminentia thalami does not appear as a distinct ridge in these dorsal sections. This is due to the large size of the primordium hippocampi in Lampetra as well as to the crowding together on account of the pressure of the buccal funnel. A section through the brain of Ichthyomyzon or Petromyzon dorsatus at this same level would show a distinct eminentia thalami. Compare figures 6 and 9.

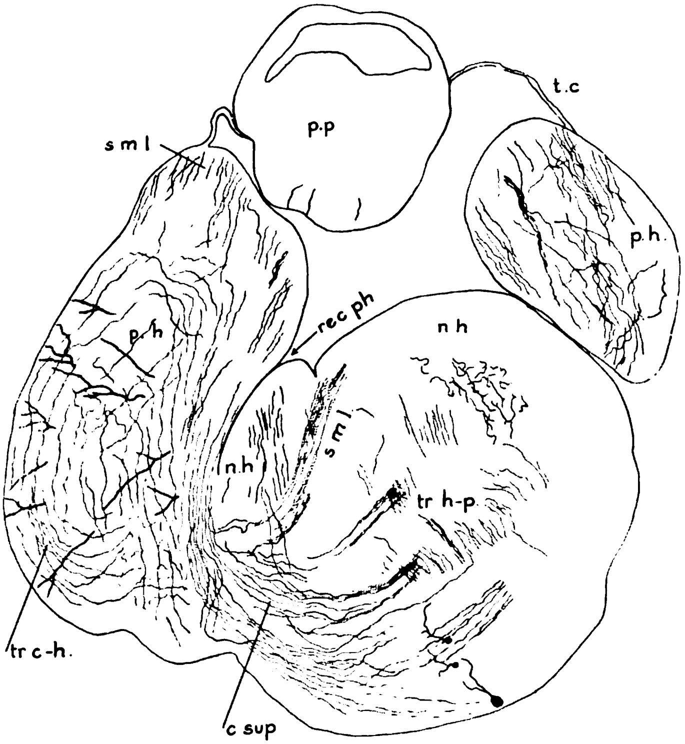


Fig. 17 The fourth section of the same series. Note the several divisions of the commissura superior (Osborn). The next three figures show that the great majority of the left stria medullaris decussates and that a large number of fibers pass through the nuclei habenulae to the opposite primordium hippocampi.

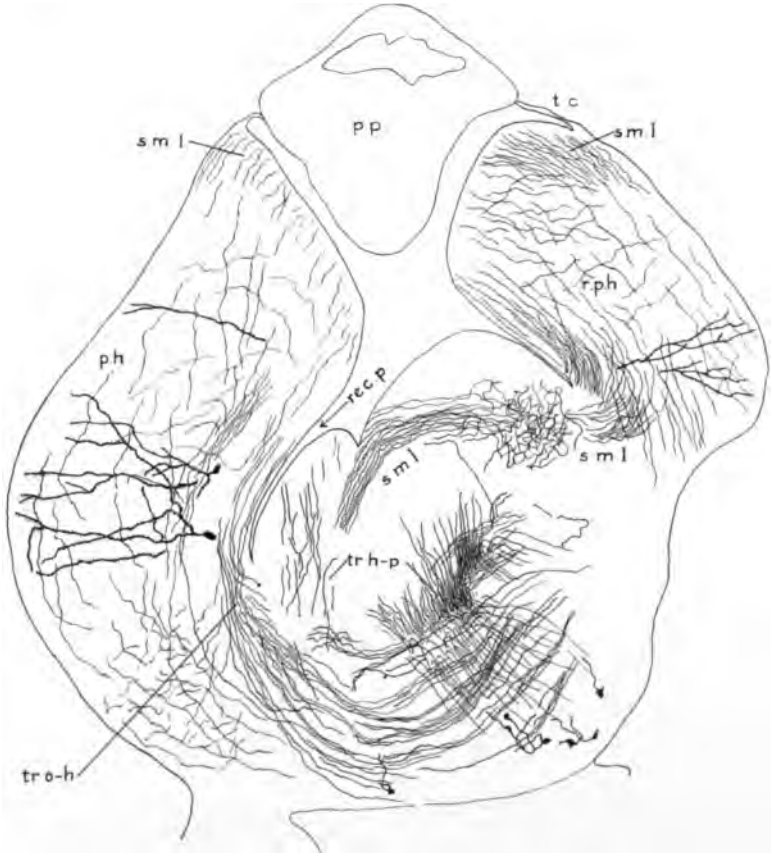


Fig. 18 The fifth section of the same series. The right primordium hippocampi shows the first stria medullaris bundle. On the left the tractus olfacto-habenularis comes up through the lower part of the primordium hippocampi and the eminentia thalami.

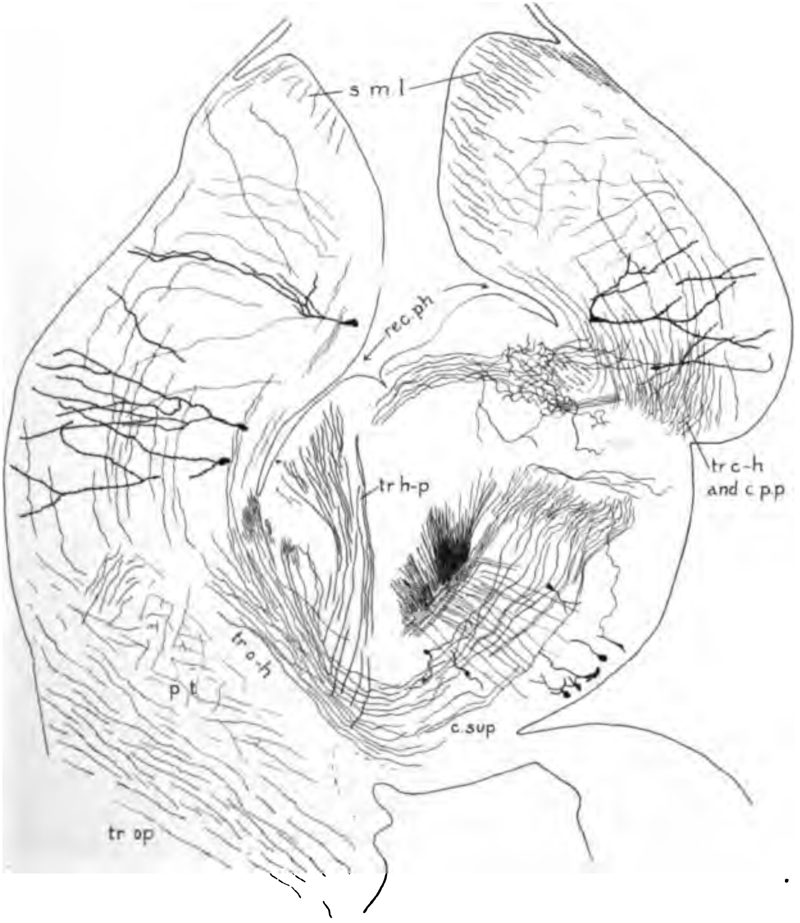


Fig. 19 The sixth section of the same series. On both sides the fibers of the tractus olfacto-habenularis lateralis and posterior appear as definite bundles which in the next sections clearly lie in the eminentia thalami.

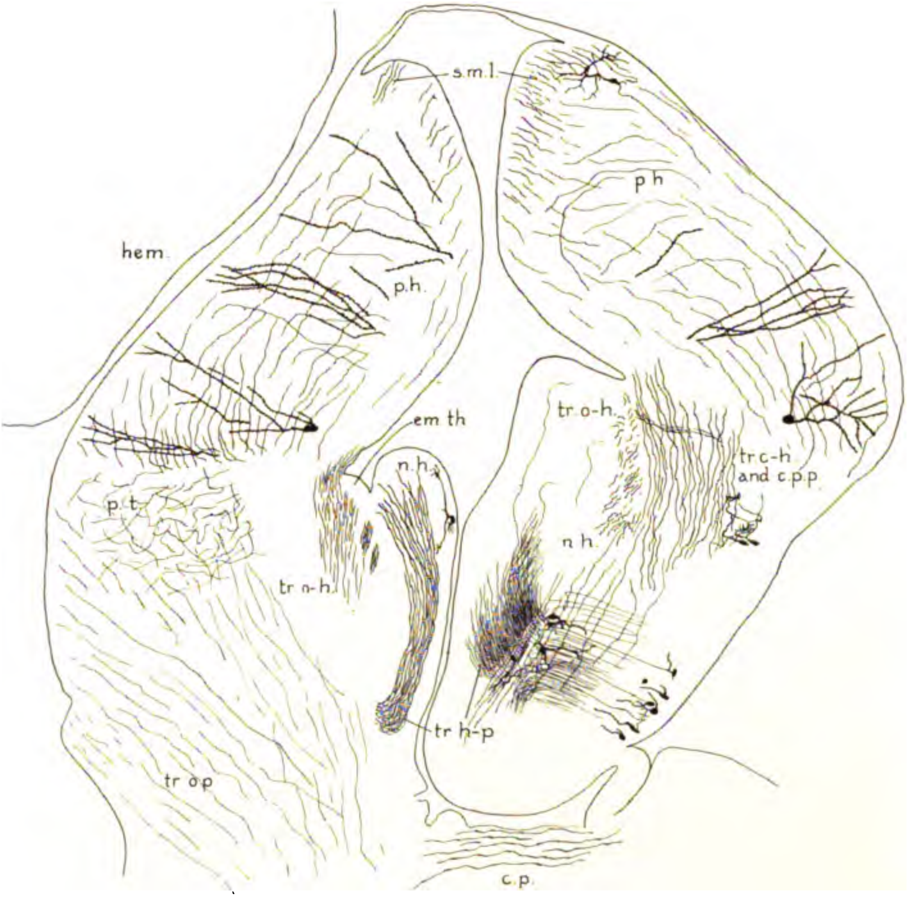


Fig. 20 The seventh section of the same series. In the rostral part of the right primordium hippocampi is seen a cell whose axone is directed toward the nucleus habenulae. Note the sharpness of the structural boundary between the primordium hippocampi and the pretectal region.

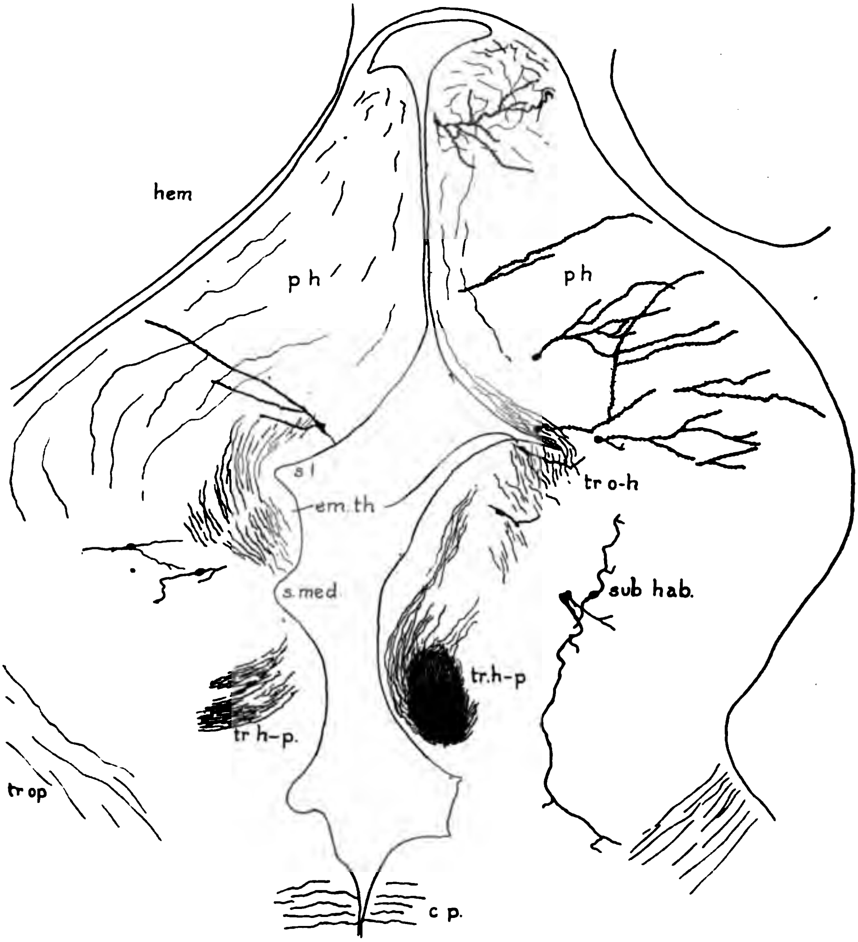


Fig. 21 The ninth section of the same series. Note the difference between the cells in the primordium hippocampi and those in the subhabenular region. The tractus olfacto-habenularis occupies a position in the eminentia thalami which is quite typical for vertebrates generally. The great number of longitudinal fibers in the primordium hippocampi are only indicated by a few lines.

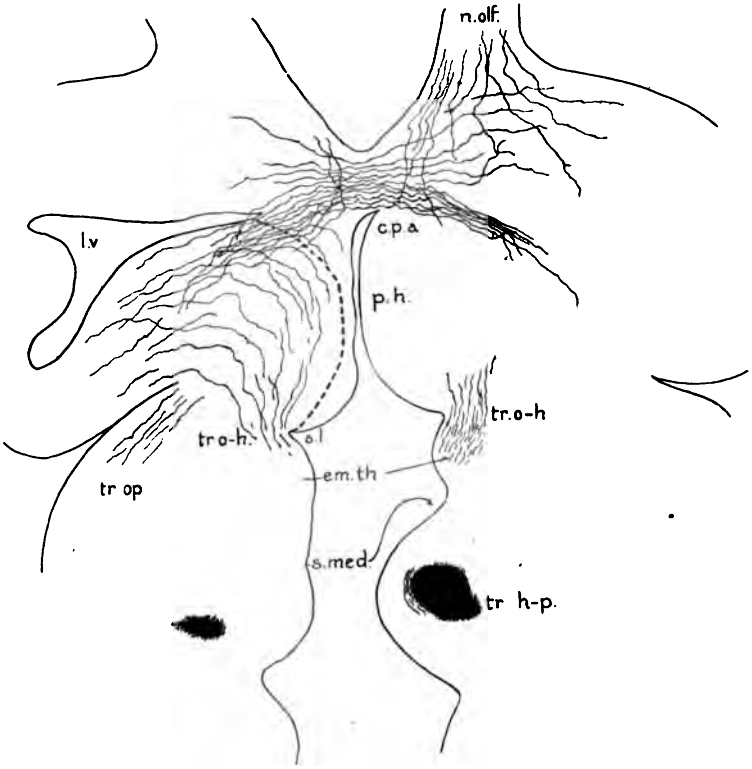
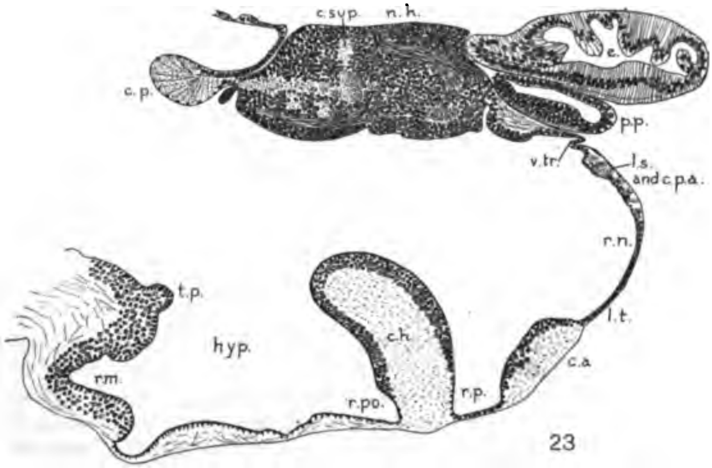


Fig. 22 The thirteenth section of the same series drawn at a lower magnification. The section passes through the anterior pallial commissure and above the interventricular foramen. The heavy broken line shows the outline of the next section ventrad, in which the sulcus limitans hippocampi passes forward to enter the foramen. Note that the primordium hippocampi is followed caudally by the eminentia thalami and this by the ridge containing the tractus habenulo-peduncularis. The sulcus medius separates these two ridges. The anterior pallial commissure in this section has only fibers connecting the hemispheres.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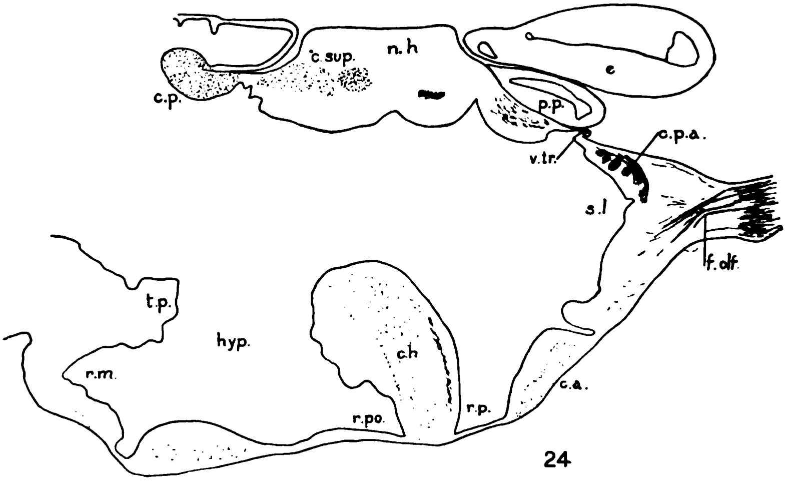
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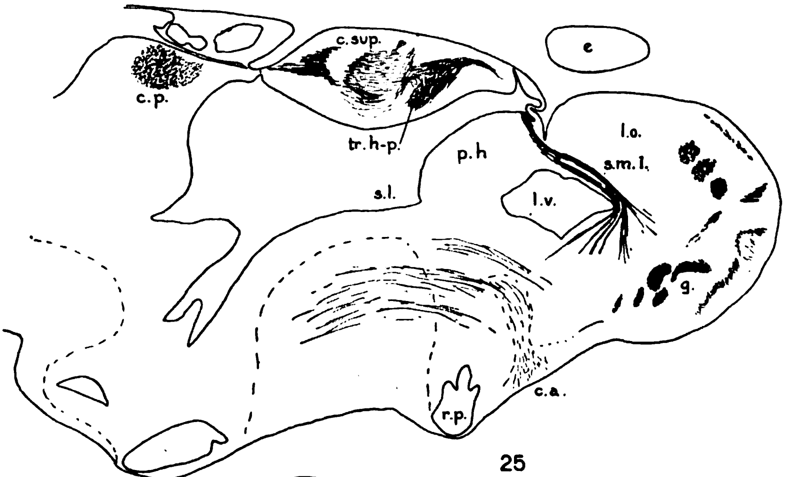
Figs. 23–27 Ammoecoetes of Petromyzon dorsatus. Five sections from the sagittal series from which the model shown in fig. 8 was reconstructed. The outlines were made with the aid of the Edinger drawing apparatus. The velum transversum is seen as a distinct fold of the tela in figures 23 to 26, but in figure 27 it joins the eminentia thalami. Figures 25 and 26 show well the course of the tract which runs up from the medial olfactory nucleus in front of the foramen interventriculare. This bundle may contain a tractus olfacto-corticalis and possibly also the fornix column. The fibers of the commissural bundles beginning with the most rostral successively turn up into the primordium hippocampi (fig. 24). The fibers which go laterally into the hemispheres are not readily seen in these sagittal sections because they are cut across. These sections show conclusively that the commissure is largely related to the primordium hippocampi.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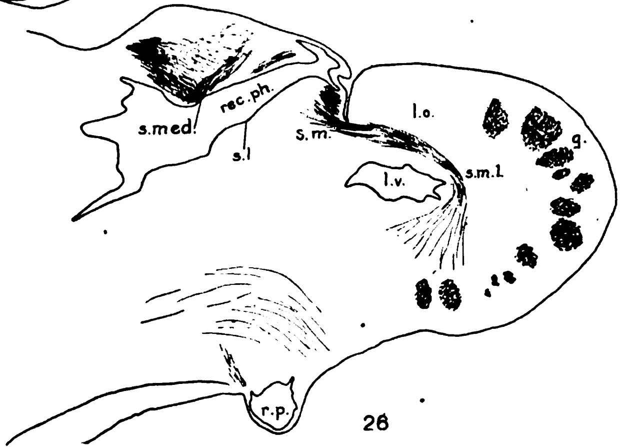
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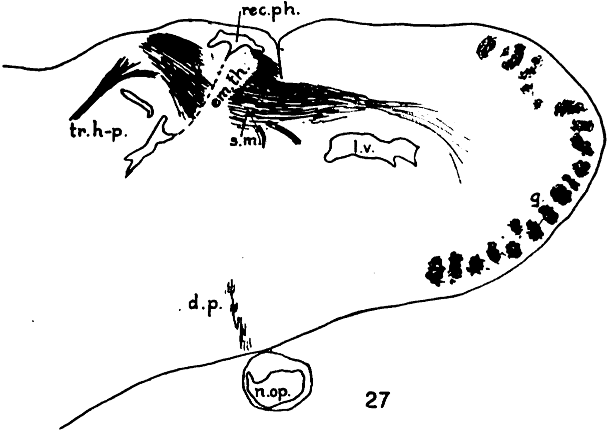
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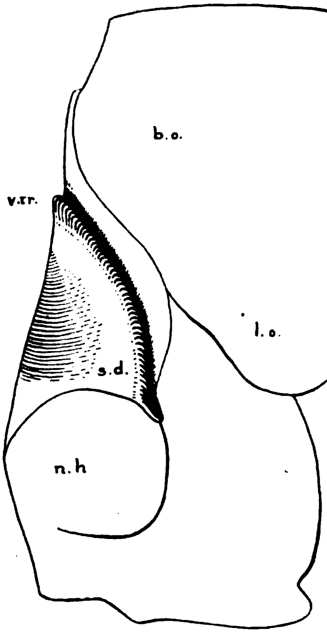

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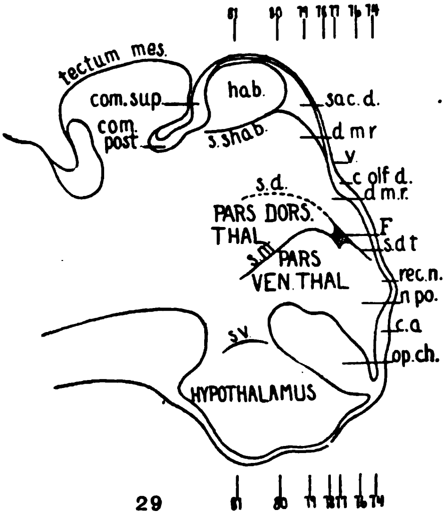

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Fig. 28 A diagram to show the disposition of the velum transversum. The outline is taken from figure 7, showing the dorsal aspect of the model of the Lampetra brain. The tela chorioidea is drawn as it would appear if the epiphysis and parapineal body were removed. The velum transversum is drawn as a fold of the tela.

Fig. 29 Professor Herrick's figure 73 reprinted for comparison with figures 6 and 9 of this paper. The figure is "a diagram reconstructed from actual sections of the cyclostome brain, Ichthyomyzon concolor."

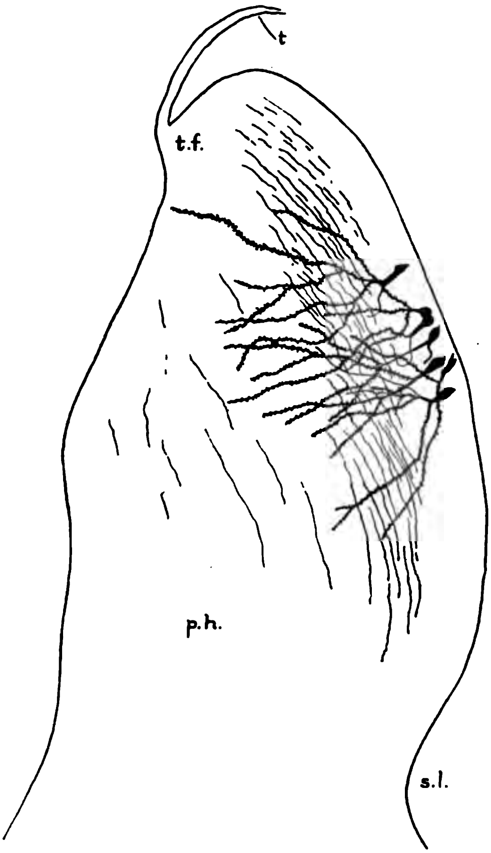


Fig. 30 Lampetra. Transverse section through the caudal third of the left primordium hippocampi. Golgi method. Most of the dendrites are longer than they appear in this figure. The distal ends of the dendrites extend nearly to the outer surface but are too intricately interlaced to be followed.

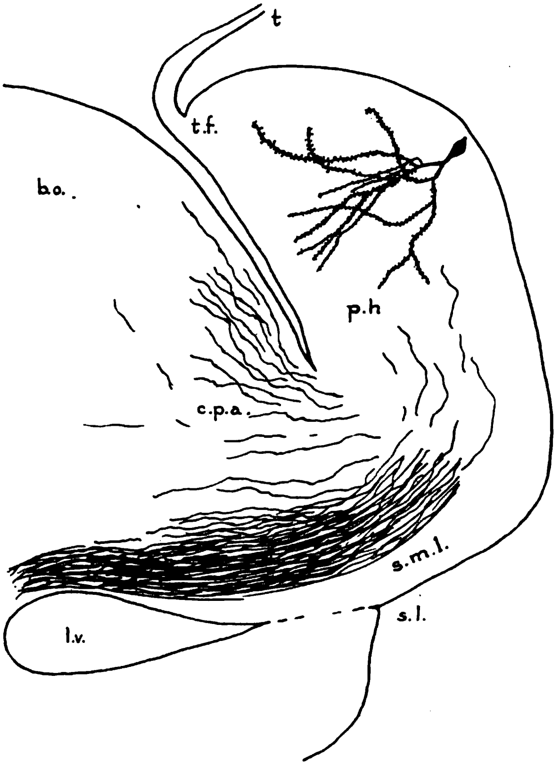


Fig. 31 Lampetra. Transverse section through the primordium hippocampi just caudal to the interventricular foramen and the anterior pallial commissure. The cells in the rostral end of the primordium are of the same type as in the caudal part (fig. 30).

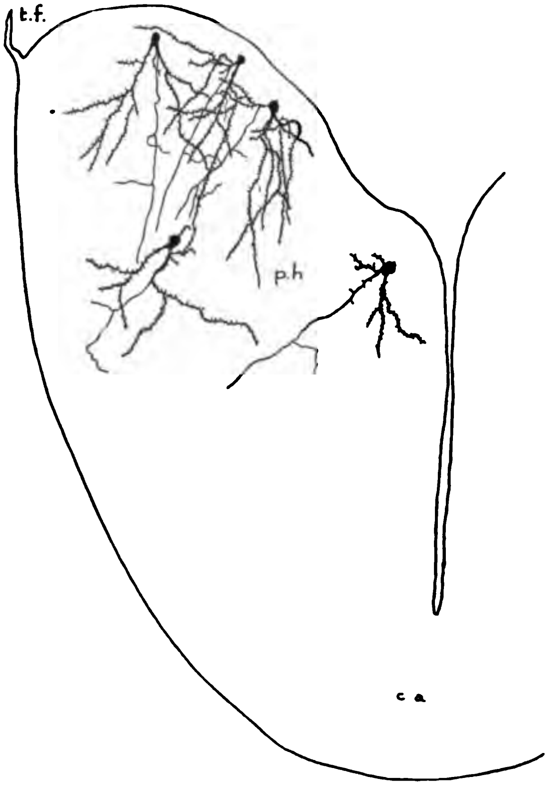


Fig. 32 Acipenser rubicundus. Five cells of the primordium hippocampi for comparsion with those of Lampetra. Note that the eversion of the forebrain in this ganoid of 30 cm. is about the same as in the adult Lampetra.

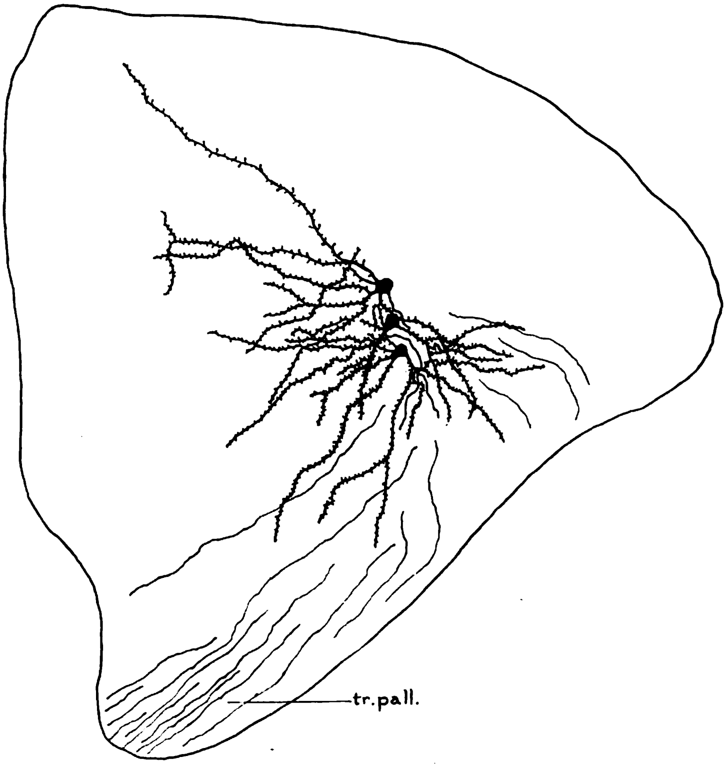


Fig. 33 Amia calva, about 15 mm. A parasagittal section near the lateral border of the forebrain, showing three cells of the primordium hippocampi and a few fibers of the tractus pallii. Golgi method.

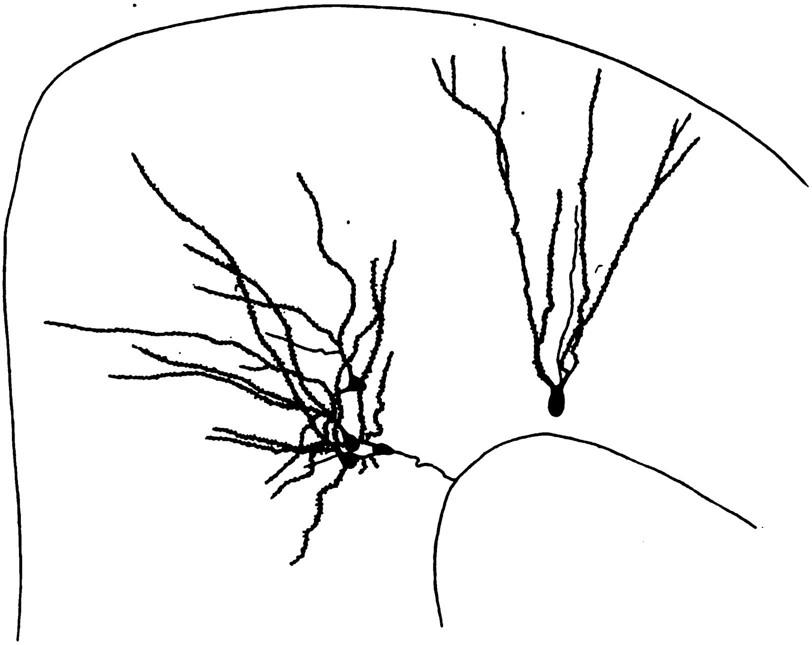


Fig. 34 Rana. Transverse section of the dorso-medial angle of the right hemisphere showing the characteristic cells of the primordium hippocampi. Golgi method.

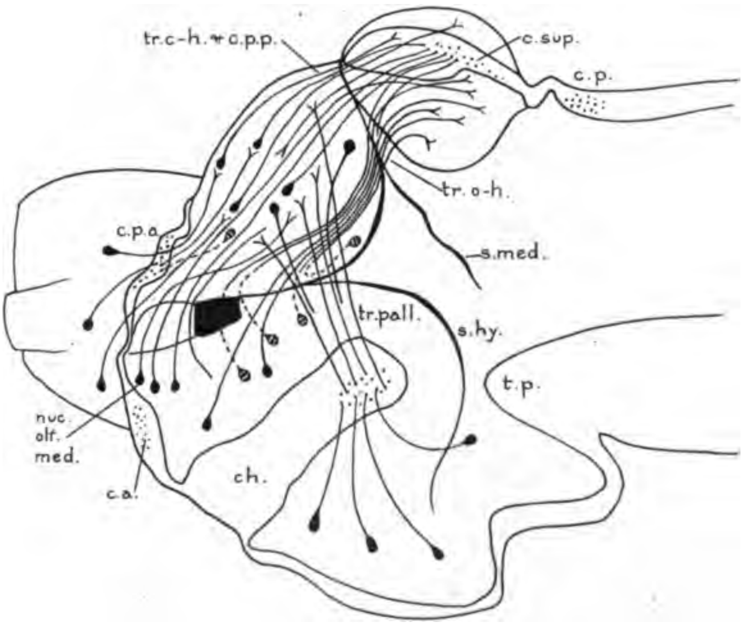
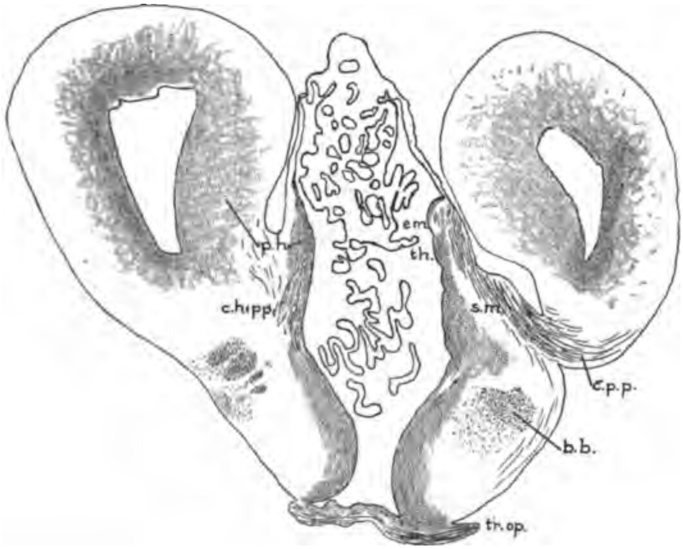


Fig. 35 Scheme of fiber tracts related to the primordium hippocampi in petromyzonts. The secondary olfactory neurones located in the caudal part of the hemisphere are represented by broken lines.



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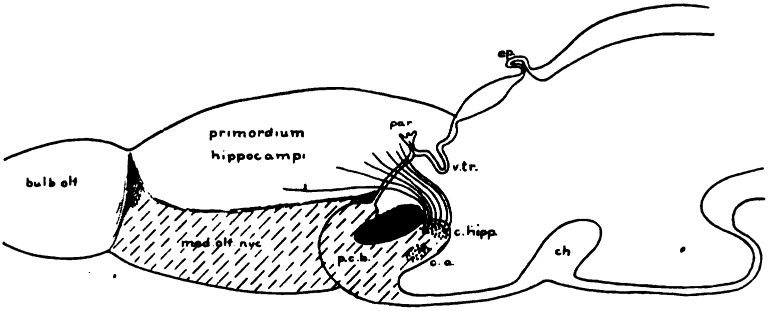

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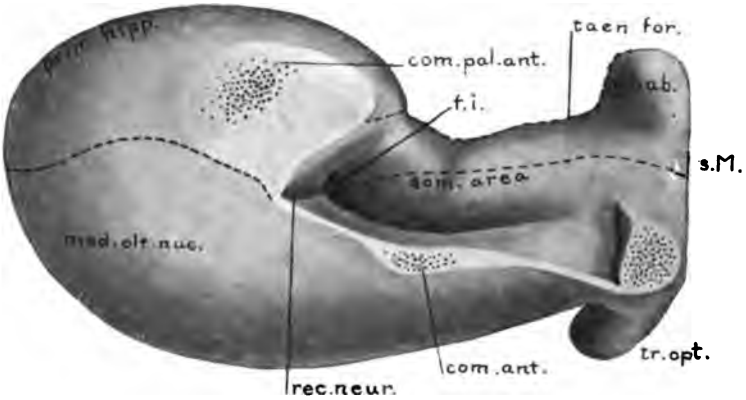
Fig. 36 Cryptobranchus allegheniensis. Transverse section through the telencephalon behind the cerebral commissures. The remnant of the primordium hippocampi in the telencephalon medium appears on the left side to the right of the letters *p.h.*

Fig. 37 Necturus. Sketch of the right half of the forebrain as seen from the medial surface. This figure is introduced merely to show the general relations of the medial zona limitans which extends rostrally from the interventricular foramen and separates the medial olfactory nucleus and primordium hippocampi. Its formation has been brought about by the evagination of the region which in the brain of cyclostomes lies rostral to the foramen. Compare figure 5.



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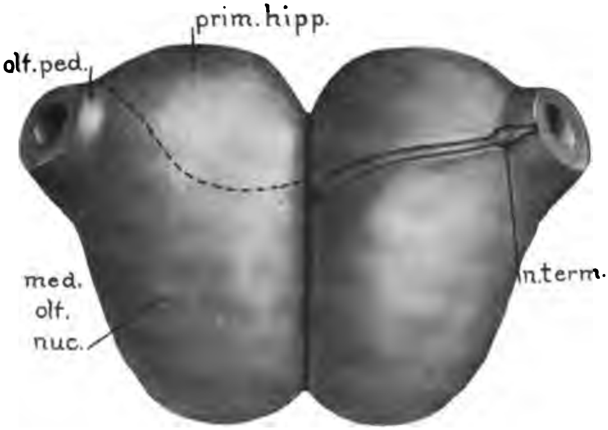

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Figs. 38 and 39 Two drawings of a clay model made to represent a simplified selachian forebrain. From Johnston '11 a. Figure 38 shows the medial surface, figure 39 the rostral surface. The broken line on these surfaces represents the medial zona limitans hippocampi. It extends from the neuroporic recess through the interventricular foramen along the medial wall of the hemisphere. Compare the figures of sections in the paper referred to. This zona limitans is homologous with the line of separation of the primordium hippocampi and medial olfactory nucleus rostral to the foramen in cyclostomes. See figures 3, 5 and 6. In figure 38, *s.M.* marks the continuity of the sulcus limitans hippocampi with the sulcus hypothalamicus, very much as in Lampetra and Ichthyomyzon. Compare figure 41.

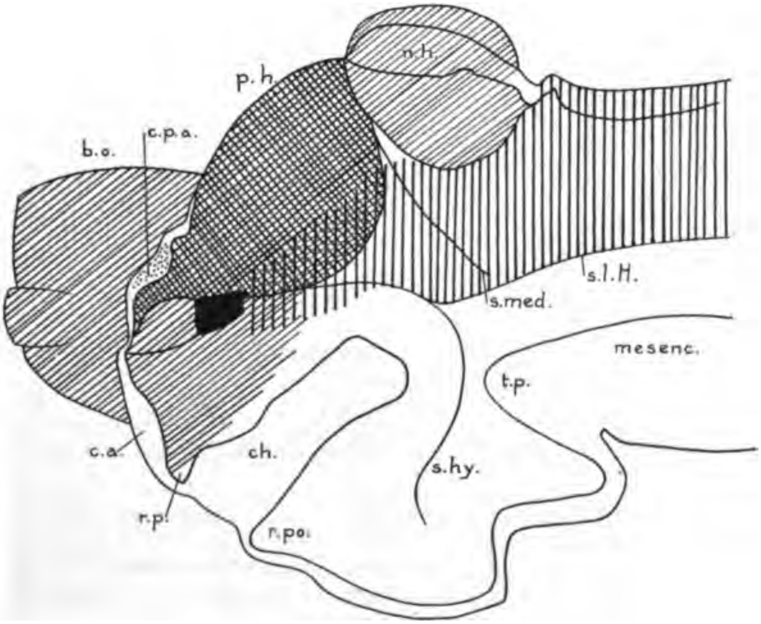

Fig. 40 Diagram of the relations of functional columns drawn on the outline of the Lampetra brain. The visceral receptive column is shaded by oblique lines, the somatic receptive column by vertical lines. The primordium hippocampi is cross-hatched with oblique lines and it is evident that it is the caudal portion of the column devoted to olfactory and gustatory functions. The lower border of the cross-hatched area in the figure is the sulcus limitans hippocampi, both rostral and caudal to the interventricular foramen.

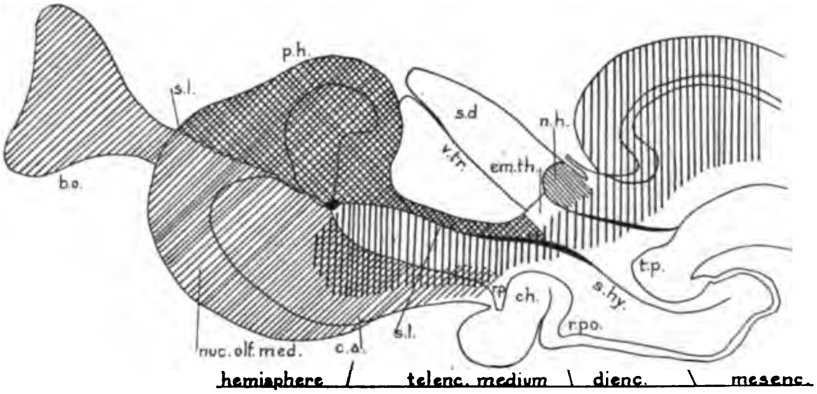

Fig. 41 Diagram similar to that of figure 40, drawn on the outline of the brain of Squalus acanthias. Drawn from the bisected brain. Owing to the somatic receptive area being situated on the lateral surface of the telencephalon, its projection on this figure overlaps the olfactory area. The medial sulcus limitans hippocampi is marked by a wavy line along the lower border of the primordium. This figure illustrates the U-shaped flexure of the visceral receptive column, the attachment of the olfactory peduncle at the base of the *U*, and the position of the somatic receptive area between the two limbs of the *U*. The axis of the brain ends in the recessus praeopticus and the great forebrain flexure involves only the dorsal columns.

Reprinted from The Anatomical Record, Vol. 6, No. 3
March, 1912

THE ANATOMY OF THE HEART OF THE INDIAN ELEPHANT

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From the Institute of Anatomy, University of Minnesota

The specimen upon which the following description is based was obtained from a small female elephant that died in this city during a spell of hot weather. The lack of facilities for dissecting so huge an animal and the obligatory haste made it impossible to study any topographical relations and was the cause of the destruction of many parts that may prove of interest. After the heart was removed it was placed into formalin and it remained there for eight months. When it was taken therefrom it was found impossible to estimate the extent of the pericardium, and the number of veins that led to the atria. It had been cut off too short to determine these points, but in other respects the specimen is in excellent condition and justifies a detailed description.

The literature is very inadequate and contains many erroneous and conflicting statements. With the exception of the three most recent articles, Vulpian et Philipeaux ('56), Watson ('71), Miall and Greenwood ('78), we find that the bibliography has mainly an historical interest. Unfortunately not all the treatises were accessible, but no effort was spared to trace all previous descriptions to their source. The literature will be discussed in the course of this article.

The general shape of the heart is like a broad short cone somewhat flattened in antero-posterior diameter.[1] The diaphragmatic face has the shape of an equilateral triangle. The auricles (auricular appendages) are very small compared with the size of the heart. This may be due to a greater degree of contraction of the auricles.

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[1] In describing the isolated heart I follow the rule adopted by most authors, namely, to imagine it with its base upwards and the interventricular septum in a sagittal plane.

A thick epicardium covers the whole heart hiding the coronary vessels and the external divisions of right and left ventricles, except near the apex where the surface is deeply indented. Watson corroborates the statement of Aelian (1765), Stukeley (1722) and Mayer ('47)[2] that the apex is distinctly bifid, but according to our specimen it would be an exaggeration to agree with these authors. The apex is formed by the left ventricle alone. A marked oedema around the coronary sulcus obliterates it in places. It is probably this curious oedema which Vulpian and Philipeaux mistake for fat, an error which I would have made had it not been for the incisions made through it. Miall and Greenwood say that the heart they dissected was perfectly destitute of fat, and that the findings of Vulpian and Philipeaux is the only instance in which any considerable quantity of this substance has been met with in any part of the elephant. The following measurements, taken after fixation in formalin, may prove of interest.

The weight of entire heart, 10.5 kilogram.

The greatest width (at a level where the coronary sulcus crosses the septum on the posterior surface), 30 cm.

The greatest length, including aortic arch, 50 cm.

The length of ventricles from coronary sulcus to apex on the posterior surface, 25 cm.

The length of ventricles from coronary sulcus to apex on the anterior surface, 32 cm.

The antero-posterior diameter, 24 cm.

These measurements bear out the statement made by previous authors, Meckel (31), Owen ('68), that the heart is broader than it is long, for if we omit the aortic arch the length would be about 28 cm. as compared with 30 cm., the width.

In order not to destroy the heart as a museum specimen and to allow of further investigation, it was cut open in the manner usually pursued by pathologists. The left side was opened by joining an incision through the aorta and the anterior wall of the ventricle to one through a right pulmonary vein and the posterior wall of the ventricle at the apex. The right side was opened by a similar triangular incision through the pulmonary orifice and inferior vena cava. In the specimen at hand the cavity of the right ventricle and conus arteriosus seems about twice as large as that of the left ventricle. This is probably due to the greater degree of contraction of the walls of the latter, and must be taken into consideration when comparing the following measurements:

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[2] After looking up the original article of Mayer I found that Watson had misquoted him. His description, with which mine agrees, is as follows: "An dem Apex Cordis sind beide Ventrikel aeusserlich durch eine tiefe Crena geschieden, so dass der Ventriculus sinister die Spitze des Herzens allein bildet. Ob es von dieser Theilung nahe der Spitze des Herzens herruehrt, dass man von Aelian bis auf Conrad Gessner dem Elephanten Zwei Herzen zuschrieb will ich dahin gestellt sein lassen."

The average thickness of the wall of the left ventricle is 5 cm. while the thickness of the right ventricle near the apex is 1.5 cm. increasing toward the pulmonary orifice to 2.3 cm. The interventricular septum measures 7 cm. in thickness.

The endocardium is grayish in color and smooth. In places it measures over 0.5 mm. in thickness and can easily be stripped off in two layers. Beneath it, the musculature of the ventricles shows a marked cross-striations of its fibers. Upon histological examination, however, it was found that these cross-striations have nothing to do with the usual cross-striations of cardiac muscle, but are merely folds caused probably by the great degree of shrinkage of the endocardium in the fixing fluid. There does not seem to be any marked difference between the cardiac muscle fibrils of the elephant and those of other mammals in respect to their structure or their size. No Purkinje fibers are visible to the naked eye in either ventricle and no histological examination was made for them.[3] Their invisibility may be due to three causes, viz., the thickness of the endocardium which hides the musculature in general, a deeper position of the Purkinje fibers and the action of the fixatives which destroys the difference in gross appearance between Purkinje fibers and heart muscle. Small bloodvessels in the endocardium were conspicuous.

The inner surface of the heart offers no marked peculiarities with the exception of the presence of numerous foramina venarum minimarum (Thebesii) throughout both atria and ventricles.

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[3] Since writing this article a description of the Purkinje fibers in the elephant's heart by Manuilow ('11) has appeared.

Ever since the time of Haller (1757), who denied their presence, there has been a discussion as to whether these foramina are the openings of actual veins or merely depressions of the endocardium. Practically all the modern text-books take the non-committal attitude that some are and others are not openings of real veins. The author has frequently attempted by injection through the coronary arteries in the pig's heart to obtain an injection of these venae minimae of the ventricles but without success. To attempt injection methods in a heart that has been thoroughly fixed as is the case with this heart of the elephant, would be perfectly futile. A positive result may indicate a rupture of a bloodvessel for it takes upwards of 300 mm. mercury pressure and a negative result may mean an occlusion by a blood-clot. However, owing to the fact that the orifice of some of these veins measures 3 mm. in diameter it was easy to determine by dissection that one was not dealing with ordinary depressions of the endocardium. They gave every appearance of being veins, but if we consider the development of the heart we must be very careful in making the statement that they actually are veins. The ventricular walls of the embryo consist of a sponge like network and into the trabeculae of this network the coronary capillaries extend. In places there is but a unicellular layer which separates the ventricular cavity from the coronary system. If there were actual intercommunication between the two we would be dealing with a sinusoid and the presence of venae minimae in the adult heart would indicate a persistence of this sinusoid. It is easy to conceive, however, of some of these trabeculae being pushed together by the growth of the ventricular cavity and not losing their endothelial covering in the course of development. These could, in the adult heart, easily simulate veins and their true nature can be determined only by injection of a fresh heart. In Bell's Anatomy (1812) we find the following statement, "Du Verney was so far engaged in this question (the presence of venae minimae), that having an opportunity of dissecting the heart of an elephant he tied up the coronary arteries and veins, washed and cleaned very thoroughly the cavities of the heart; and then tried, by squeezing, and all kinds of methods, to make that blood which was tied up in the coronary arteries and veins exude upon the inner surface of the heart, but with no effect." In a previous paragraph they condemn the crude methods used by Vieussens, Thebesius and others who believe these openings to be openings of true veins.

The introduction of a needle in various parts of the septa atriorum and ventriculorum failed to reveal the presence of an os cordis, a fact which is surprising because its presence has always been associated with all large hearts, and a number of recent text-books state that it is present in the elephant's heart. This question has been the subject of a great deal of discussion ever since the time of Galen. Galen states that he found a bone in the heart of an elephant and kept it in his possession for a number of years. Moulin (1682), Blair (1708), and Perrault (1734) did not find it, attributing it to the youth of the animal they had under observation. Camper (1802), however, argued that in young animals one would at least find cartilage, as he did in a calf six weeks old, which he had dissected for the purpose of settling this question. In the elephant's heart he found neither bone nor cartilage. This seems to be the case in our specimen. It may well be that Galen had before him a sclerosis of a coronary artery. Mayer ('47) finds a "Knorpelstreif von 10 Linien am Ostium arteriosum des linken Ventrikels." The position he gives is too vague to comment upon. The size of the heart has evidently nothing to do with the presence or absence of an os cordis. My histological examinations have never failed to reveal bone tissue in the human and the dog's heart, and fibro-cartilage in smaller animals such as the rabbit and guinea pig. The lack of a bone in the heart of the elephant is due to poor development of the trigona fibrosa. Some of our leading text-books on human anatomy fail to recognize these trigones although they never fail to mention so unimportant a structure as the tuberculem venosum (Loweri) which is absent in the human heart. The trigona fibrosa (B.N.A.) are two masses of connective tissue which lie between the aorta and the orifice of the mitral valve. The left is near the periphery of the heart and the right in the triangle bounded by the aorta anteriorly and the mitral and tricuspid ring on the left and right side respectively. It is the right trigonum which is the primary seat of the os cordis. A second os may develop in the left trigonum and may fuse with the right bone. In the elephant there is a slight shifting of position, due probably to the greater development of the conus arteriosus, so that the mitral ring does not lie on the same plane as the aorta but lower down and the right ring lies higher up. Posterior to the root of the aorta we have atrial musculature. This torsion was recognized by Miall and Greenwood who make the following statement:

> The right side of the heart is, so to speak, rotated upon the centre of the left; the right auricle being thrown to the dorsal surface of the heart, while the principal axis of the right side, passing directly through the auriculo-ventricular orifice, is inclined upwards, instead of lying nearly horizontal, as does that of the left side in the animal as it stands. The inferior face (anterior of man) gives no indication of this tilting of the right side, except that the right auricle is displaced; the two ventricles meet along a straight line and divide the lower surface nearly equally.

The cavity of the left ventricle is distinctly conical in shape but the lower two-thirds is very irregular owing to the large trabeculae and columnae carneae which are arranged mostly longitudinally to the axis of the heart. The upper third of the septal wall is comparatively smooth. There are two large papillary muscles controlling the mitral valve, the anterior measuring 5 cm. in diameter at its base and the posterior 2 cm. There are three distinct sets of cords or tendons attached to the mitral valve such as Kürschner ('44) has described for the human heart. The first set is fixed to the attached margin of the valve, the second to the middle, and the third to the free marginal edge.

The right ventricle is divided into two parts by the crista and trabecula supraventricularis.[4] The upper or anterior part, the conus arteriosus, ought rightly to be designated as the fifth chamber of the heart. A comparative study of the mammalian heart soon reveals the necessity of dropping the old division of the heart into four chambers, which may have had its didactic advantages, and of adding the conus as a fifth chamber. Both in its anatomy and physiology is the conus arteriosus somewhat distinct from the right ventricle and much confusion would be avoided by using the term right ventricle in its restricted sense. The right ventricle of the elephant has a rather small cavity, which at the lower third of the septum and the entire outer wall is traversed by large trabeculae and columnae carneae. There are three papillary muscles, but their peculiar arrangement can be understood only after a study of the conus has been made.

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[4] In J. H. H. Bull., vol. 20, p. 172, 1909, I suggested the name trabecula supraventricularis to that portion of the crista which stretches across the cavity of the ventricle and corresponds to the moderator band of English authors and the bandelette ansiform of Poirier. The term moderator band was also used for all strands which stretched across the cavity of either ventricle, although in its restricted sense it applied only to the band which is constant in the sheep and pig.

The conus arteriosus of the elephant seems to have the greatest capacity of any chamber of the heart, and differs from that of other hearts in that it has a large septal wall. We usually find the conus consisting of a very narrow septal and a very extensive free wall which give it its typical external shape from which its name is derived. In the elephant the increase of the septal wall is at the expense of the right ventricle whose septal area is correspondingly reduced. It is this difference of growth that has brought about the obliteration of the undefended space and the trigona fibrosa as before mentioned.

To understand the peculiarity of the position of the papillary muscles in the right ventricle of the elephant's heart it will be necessary to call attention to the condition usually found in other hearts. Detailed description will be found in papers by King ('37) and by Retzer ('09). The number of papillary muscles may vary from one to six and is inversely proportional to the size of the papillary muscles. The larger the anterior papillary muscles the fewer the number. King divided the mammalian hearts into four series according to the presence or absence of a moderator band. These series are typically represented by I, Rodent, Canine and Marsupial animals; II, Feline; III, Quadrumana and Human; IV, Ungulates. The trabeculae supraventricularis (moderator band) which always contains the right branch of the conductive-system (Reizleitungs system of Tawara) is found constantly in series IV where the anterior or large papillary muscle that controls the greater part of the free cusps is situated on the free wall. In Quadrumana and Human this papillary muscle is situated about midway between the septal and free wall, its base resting upon a complex network of trabeculae. In series II the tendency is for this papillary muscle to arise from the septal wall, a condition which is almost invariable in series I. The number of papillary muscles seem to increase from series IV to series I. In all of these series the septal cusp is controlled either by numerous small papillary muscles and tendons arising from the septal wall itself or by a somewhat larger one situated near the posterior edge of the septum. The anterior papillary muscle is always the largest and is the most important for the control of the free cusps.

In the elephant we find the anterior papillary muscle controlling the septal leaflet while the two others control the free cusps. Their position is correspondingly on the septal and free walls. Such a condition is unknown to me nor does King mention it in his study of 300 hearts. The base of the anterior papillary muscle is situated on the septal wall immediately posterior to the crista supraventricularis and its apex points in the direction of the coronary sinus. It controls the entire septal cusp with the exception of its most anterior edge which is held by tendons that arise out of the septum. These tendons are constant structures in all mammalian hearts, and form one of the landmarks of division between ventricle and conus. In a few instances the author has found these tendons arising from a small papillary muscle whose fibers could be traced to a region near the pulmonary orifice. The muscle is of considerable size though not as large as the two others. Its position may be accounted for in the following manner. Usually the mesial cusp of the tricuspid has a broad base of attachment, many small tendons arising from the septum attaching to it throughout its width. The cusp in these instances cannot extend far into the cavity at the time of systole and the two other cusps are brought during contraction close enough to prevent regurgitation. In the elephant the size of the tricuspid orifice necessitates larger cusps (they measure 6.5 cm. in width) and complete action of them all. We find correspondingly a septal cusp of a very narrow margin of attachment. The ventricle is however so short that it would be impossible for it to accommodate a papillary muscle long enough to control the mesial cusp, unless this muscles should act at an angle and thus reduce the necessary degree of contraction. Furthermore a large papillary muscle on the septum below the tricuspid orifice would be quite an obstacle and occlude a great part of the cavity. We find therefore the papillary muscle situated almost in the conus flattened agains the septal wall. This papillary muscle must therefore be considered as the homolog of the posterior papillary muscle in other animals, the change of its position being due to the unusual development of the conus and a corresponding decrease of the right ventricle. The other papillary muscles of equal size and slightly larger than the first one mentioned are situated on the free wall. The more anterior rests in part on the crista supraventricularis. They control the two free cusps. According to Miall and Greenwood the tricuspid may have one or two small additional cusps. In our specimen there is but one additional, between the anterior and septal cusp.

The aorta and pulmonary arteries do not present any marked peculiarities except the lack of a nodulus valvulae semilunaris (Arantii) in the cusps of pulmonary. The base of the valve is not attached to the wall of the vessel but to the circular muscle which surrounds both the pulmonary and aortic orifice. We find this condition in all hearts to a greater or less degree and is a fact not generally recognized. These circular muscles are the sphincters of the aorta and pulmonary artery and aid in the closure of the valves. The following measurements were taken: The diameter of the aorta 9.0 mm., thickness of wall 1.5; the diameter of the p. a. 6.5 mm., thickness of wall 1.0; the diameter of the cor. art. 1.0 mm., thickness of wall 0.2. At the sinus of Valsava the thickness of the walls of both aorta and pulmonary artery is reduced by one-half. There are two coronary arteries, not one, as has been repeatedly quoted from Camper, and they are situated at the upper edge of the sinus of Valsalva. The vessels are joined together by loose areolar tissue and can easily be separated to their origin.

The atria are very small but this may be attributed to shrinkage. The walls are thin but in the auricles they are strengthened by many pectinate muscles. As stated above the veins were cut so near the heart that it is impossible to analyze the conditions thoroughly.

The following quotation is taken from Miall and Greenwood:

> Left Auricle—The entry of the pulmonary veins seems to vary. Vulpian and Philipeaux found two openings into the auricle—a small internal and a large external, which latter received three of the pulmonary veins. Dr. Watson describes four separate openings. In our dissection there was a large central orifice and a smaller one on each side—one internal and the other external; but the external vein was not altogether clear of the central one. A thin ridge upon the internal surface of the auricle separates the central from the internal orifice. The veins enter a thin and membranous sinus, which is slightly separated from the rest of the auricle by a prominent fleshy ridge. Part of the edge of this ridge forms the 'valvular structure' noted by Dr. Watson.

Very distinct in the left atrium is the semilunar fold which stands out like a crescentic valve. In its broadest portion it measures 1.5 cm. from the edge to its attachment. The fovea ovalis lies 3 cm. posterior to it, and is thin enough to be translucent.

The right atrium presents conditions that are very interesting from the view point of development. There are three vessels that empty into the right side of the heart, the inferior vena cava and the right and left superior venae cavae. The coronary veins empty into the left superior vena cava, where it is generally spoken of as the coronary sinus. The positions of entry of these vessels do not differ markedly from other hearts, except that there seems to be a narrower interatrial septum in the elephant so that the opening of the right superior vena cava lies nearer to the inferior than is usually the case.

The tuberculum intervenosum (Loweri) was looked for but, as usual, in vain. I have, so far, carefully examined the hearts of man, dog, cat, rabbit, guinea pig, bear, sheep, rat, and opossum, which is a sufficiently representative group to say with absolute assurance that there is no such thing as a *tuberculum* intervenosum. By a tuberculum we mean a tuberlike or knoblike projection. It is in this sense that I deny its presence, and it was, therefore, a great satisfaction to find the following quotation from Haller, "Id tuberculum cupide receptum est, ut fere fit, ab iis scriptoribus quibus occasio ad propria experimenta nulla est, deinde etiam ab iis qui tandem omnino in corporibus humanis dissecandis se exercuerunt." It would be too much of a digression at this point to go into the details of development but suffice it to say that the tuberculum intervenosum never does exist at any stage of development, and Pohlman ('09) has shown that there is no physiological necessity for it. In the developing heart it can be clearly seen that the tuberculum intervenosum is nothing but a ridge which is the continuation of the limbus fossae ovalis, and it depends upon the size and position of the limbus whether or not there is any 'crista' intervenosum on the upper outer wall. In the elephant's heart the superior vena cava is separated from the inferior by the limbus fossae ovalis, which lies immediately posterior to it and slightly bulges the septal wall inward, i.e., into the cavity of the right atrium, and by the fossa ovalis itself. The posterior margin of the fossa ovalis lies really within the inferior vena cava.

It is unfortunate that the specimen is so imperfect that the extent of the valves cannot be definitely determined. Miall and Greenwood make the following statement:

> A sigmoid valve passes from the external side of the right anterior cava, adjacent to the appendix, curves round the ventral side of the orifice, and is then continued as a long membranous ridge of slight projection to the basal or anterior side of the posterior cava; it then crosses that opening on its ventral margin, becoming somewhat more prominent, and serving as a proper valve to the posterior cava; finally, it gradually disappears along the base of attachment of the Eustachian valve. This agrees tolerably well with the description of Vulpian and Philipeaux; and with Dr. Watson's figure, though in the text of his description he says that the valve passed round the upper margin of the right anterior cava. A large Eustachian valve separates the posterior from the left anterior cava. The great coronary vein opens into the left anterior cava under cover of a pectinated muscle.

What remains of my specimen bears out the description as regards the position of the valve of the superior vena cava and it seems likely that it is continuous with the valve of the inferior vena cava, but the statement that "it gradually disappears along the base of attachment of the Eustachian valve" demands some explanation.

This entire valve and fold is the persistent right venous valve which in the young foetus stretches as a crescentic fold from the right side of the superior vena cava along the upper wall of the atrium, thence along the right side of the opening of the inferior vena cava to disappear in the septum intermedium of His. As the coronary sinus develops the lower end of this valve forms the valve of the coronary sinus (Thebesii). The persistent part of the valve near the orifice of the inferior vena cava becomes the Eustachian valve. Owen states "The right auricle in the Rhinoceros, as in most Ungulates, has but one precaval orifice, and shows no valve at the termination of either the postcaval or coronary veins; the contrast presented by the elephant, in this respect, is significant." Unfortunately Owen fails to define the contrast and one can but assume that the elephant has two precaval orifices and valves guarding all the openings. Cuvier ('39) says (Meckel's translation) p. 41 "Beim Elephanten is diese (Eustachische) Klappe spiral förmig gewunden und geht längs der oberen Wand in das hintere und linke Ende einer anderen breiten und halbmondförmigen Klappe über, welche die Oeffnung der vorderen rechten Hohlvene von der Höhle des öhrformigen Anhanges scheidet." Wolff, according to Meckel in Cuvier's Vorlesungen, IV, p. 36, and Meckel himself have found instances where in absence of a Thebesian valve the Eustachian extended so far down so as to cover the opening of the coronary vein. From this it is evident that these older authors called the Eustachian valve a different structure than is understood by that name today. Search through the literature revealed the fact that the Eustachian valve was formerly called valvula forminis ovalis anterior (Bock, ('50), Soemmering ('41). That the Thebesian valve is but a continuation of the lower end of the Eustachian is a discovery that we owe to His and Born.

What then did Miall and Greenwood mean by "A large Eustachian valve separates the posterior from the left anterior cava?"

The valve they have reference to is a very prominent structure in our specimen. It is a fleshy valve, with a straight edge, about 4 cm. in length and separates the opening of the inferior posterior vena cava from the left anterior just as is stated by these authors. It can readily be seen that this is the homolog of the fold that is found in all the animals that have a persistent left superior vena cava. Marshall ('50) was the first to call attention to the fact that the Thebesian valve is always absent in the hearts of those animals which have a persistent left superior vena cava or left azygos emptying into the heart. Watson thinks that the fold that he found in the elephants heart is a Thebesian valve and concludes therefore that it forms a marked deviation from the rule as formulated by Marshall. Born ('89) describes this fold in the rabbit embryo and calls attention to the fact that it is not to be considered a Thebesian valve. A study of the developing heart of the pig reveals the fact that the lower end of the right venous valve aborts while in the human it grows over this same fold which is so prominent in the elephant, pig, sheep, rabbit and other animals. It is really not a valve but a fold that has grown in between the sinus (left superior vena cava) and the inferior vena cava. This fold has not been recognized by previous authors and will be discussed at length in another article.

Although the terminology differs we find most authors agreeing that the valve of the posterior vena cava is continuous with that of the anterior. These valves are the remains of the right venous valve of the embryo. It is interesting to note in this connection that the crista terminalis from which the pectinate muscles take their origin is present in the elephant but about 2 cm. to the right and below the valve guarding the right superior vena cava. It needs a further study to determine whether the crista terminalis really is the boundary between the primitive sinus and the right atrium. This idea has its foundation upon the theory that the right venous valve terminates on the crista terminalis, a theory which is not substantiated by the condition in the elephant's heart just described nor by any stage of development of the embryo pig's heart.

The coronary veins, two in number, empty into the left superior vena cava, their openings protected by valves. The pectinate muscle that Miall and Greenwood mentioned is also present.

The conductive system is visible in the right ventricle as a light colored strand about 3 mm. in diameter and lying along the upper border of the crista and trabecula supraventricularis. Its course is superficial in the ventricle but at the atrio-ventricular junction and in the atrium itself it lies embedded in musculature. Here also it has a different appearance and character. While in the ventricle it is a glistening white strand when dissected out and elastic, in the atrium it is dark red, tough and irregular in outline. This is evidently the nodal point (Knoten of Tawara). It measures about 1.5 cm. in diameter and 0.5 cm. in thickness and lies 2 cm. below the edge of the sinus fold and 4 cm. from the junction of mesial and anterior leaflet of the tricuspid. The left branch seemed at first to be wanting but after painstaking search its presence was established. As previously stated there are no Purkinje fibers visible in the ventricle and the inverted V-shaped strand of the left branch of the conductive system that is so constant in all other mammals as far as we know, is not visible nor did dissection reveal it. The left branch leaves the nodal point to the left of the right branch deeply embedded in the myocardium and soon after seems to divide into two branches whose course in the ventricle could not be established without destruction of the specimen. At the level of the aortic cusp it was deeply embedded in the ventricular musculature.

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Reprinted from THE AMERICAN JOURNAL OF ANATOMY, VOL. 14, No. 3
March, 1913

THE DEVELOPMENT OF THE ELASMOBRANCH LIVER

I. THE EARLY DEVELOPMENT OF THE LIVER

II. THE DEVELOPMENT OF THE LIVER DUCTS AND GALL-BLADDER

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FIFTY-FOUR FIGURES

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I. INTRODUCTION

The development of the elasmobranch liver offers many problems of interest not only for themselves, but because of their bearing upon questions regarding the structure of this organ in the vertebrates in general. The hepatic duct system shows here in its earlier stages a number of characters which are masked in forms which are more complicated or which undergo a more rapid development and in later stages the organ exhibits a number of peculiarities of interest in the light of recent work on the relation of parenchymous to vascular structures.

The present paper, based upon a study of an extensive series of embryos of Squalus acanthias[1] gives an account of the early development of the liver and the history of the principal liver ducts and the gall bladder. In a later section it is hoped to give an account of the development of the smaller rami of these ducts and of the hepatic parenchyma.

II. LITERATURE

At this point I shall review only briefly the literature of the general development of the elasmobranch liver. Particular points are considered in more detail in the separate sections and reviews of the literature by Brachet ('97), Choronschitzky ('00), Piper ('02), and Weber ('03) already cover a part of the subject.

In common with so many points in selachian embryology there was but little knowledge of the development of the liver until the researches of Francis Balfour. Rathke ('27) published an account of several selachian embryos including one of Squalus mustelus (Mustelus canis?) of an approximate length of 45 mm., in which he described the division of the liver into an anterior mass and two posterior lobes and traced the course of the ductus choledochus to the intestine. He stated that the gall bladder was absent in this specimen as well as in an older one of Squalus canicula (Scyllium canicula?). At such a stage this structure is, in fact, embedded in the liver substance and not visible externally. Rathke observed the gall bladder however in an embryo of Squalus mustelus 7 inches 2 lines in length, and traced the course of the vitelline veins to the liver and followed their ramifications in this organ to their final connections with the sinus venosus.

Franz Leydig ('52) in his "Beitrage zur microskopischen Anatomie und Entwicklungsgeschichte der Rochen und Hai" figured a cleared embryo of Squalus acanthias approximately 18 mm. in length in which the liver is pictured as a dark irregular mass lying just posterior to the heart. He described the cells of this organ as arranged in lobules and containing numerous fat droplets embedded in a homogeneous ground substance. He also traced the course of the omphalo-mesenteric veins through the liver.

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[1] This material consisted of sectioned embryos of Squalus acanthias from 3 to 86 mm. in length as well as several specimens of the same species in the 'pup' stage, and a few large embryos of Mustelus laevis and Squalus sucklii (?). In a large part these specimens were from the Harvard Embryological Collection, and I wish to express here my thanks to Dr. Charles S. Minot for their use for a prolonged period, as well as for the privileges of his laboratory during a part of the time while this study was in progress. Four specimens were also from the embryological collection of the University of Kansas. For their use I am indebted to Dr. C. E. McClung.

Balfour ('76) working with Scyllium, Pristiurus and Torpedo, described the liver as arising in Stage I, when forty-eight pairs of somites and three pairs of gill pouches are present, as a ventral outgrowth from the 'duodenum' directly anterior to the umbilical canal. This outpouching gives off at once two lateral diverticula which are the rudimentary lobes of the liver, while the remainder of the original ventral median pouch forms the gall bladder and ductus choledochus. The hepatic tubule diverticula appear as hollow buds by stage K, and increasing rapidly both in length and number soon anastomose forming a regular network. In the course of these changes the lumina of the tubules become much reduced in size. The gall bladder arises as dilatation of the anterior end of the median pouch and its duct joining with the hepatic ducts forms the ductus choledochus.

Hammar ('93) figured and described the first series of reconstructions of the selachian liver. These are of embryos of Torpedo ocellata, the first of forty segments, and the remainder, 9, 11, 15 and 18 mm. long respectively. These specimens correspond roughly to Balfour's stages J, K, L, M, and O. From the study of these models Hammar concludes that the liver arises primarily from three diverticula, two lateral and one median in position, and not from a single median and ventral pouch as stated by Balfour. He also considered the liver proper to arise from the lateral diverticula, the median giving rise to the gall bladder and its duct only. He noted further that there was a twisting of the fore gut from left to right, a point apparently overlooked by other observers. The account of the formation of the common bile duct and gall bladder is fairly complete, but he traced the hepatic ducts no farther than their entrance into the lateral masses of hepatic trabeculae.

In a later paper of a more general nature on the early developments of the liver, Hammar ('97) expresses his views in regard to the origin of the selachian liver as follows:

> Bei den Selachiern wird ebenfalls eine unter dem Herzen hervorragende stufenähnliche Leberfalte gebildet, an deren cranialen Rand schon frühzeitig *zwei bilateral-symmetrische Divertikel* auftreten—Zwischen diesen beiden Divertikeln und beinahe gleichzeitig mit ihnen entsteht als eine cranioventrale Verlängerung der Leberfalte noch ein drittes medianes Divertikel, aus welchen die Gallenblase und Gallenblasengang hervorgehn.[2]

I quote Hammar at length for he holds a view somewhat different from that accepted by most investigators and one which this paper will in part confirm.

Laguesse ('94) gave a brief account of the development of the liver in Squalus acanthias in connection with his study of the pancreas in this form. He states that the liver arises a little later than the pancreas, a point which has since been disproven, and although in possession of younger embryos, he apparently first observed the organ in an embryo 8 mm. in length, where it appeared as a thick walled ventral pouch extending from the primitive sinus venosus to the anterior wall of the yolk-stalk. An embryo of 9 mm. length showed the formation of the lateral diverticula. At 16 mm. the buds of the hepatic tubules had appeared and at 19 mm. they were fused together, forming the typical net-work of hepatic trabeculae so often described. Laguesse emphasizes the late appearance of the gall bladder as a structure distinctly separated from the hepatic anlage proper. This seems to me to be a point of much importance which apparently has not been recognized by other workers in this field, with the exception perhaps of Hammar.

Brachet ('96) devoted the first part of his contribution to the development of the liver and pancreas to the selachian liver as represented by Torpedo ocellata. Like Hammar he presented a series of reconstructions corresponding to Balfour's stages J, K and L. The two main points in this paper consist of, first, an affirmation of Balfour's statement that the liver arises between the anterior intestinal portal and the sinus venosus as a single median ventral pouch from which the lateral pouches secondarily arise, and second, the recognition of the fact that the medita pouch which can be distinguished when the liver reaches the rinlobed stage consists of two portions, named in accordance with the nomenclature suggested by Goeppert ('93) the 'pars hepatica' which lies anteriorly and joins with the two lateral pouches in forming the true hepatic parenchyma, and a posterior portion called the 'pars cystica' which forms the gall bladder and the cystic and common bile ducts. Brachet followed the history of the latter structures in detail, but gives little information as to the history of the hepatic ducts, although he recognized that they were formed from the bodies of the lateral pouches and considered that these pouches were reduced in caliber in the course of their transformations. In the Ergebnisse for the same year Brachet ('97) repeats his conclusions and summarizes the preceding literature since the time of Balfour.

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[2] The italics are the author's.

Rückert's work ('96) on the development of the spiral valve in Pristiurus is illustrated by three reconstructions, two of which include the gall bladder and ductus choledochus and illustrate well the forward migration of the former structure. Rückert describes the migration of the ostium of the ductus choledochus in relation to the vitelline duct and its movement from left to right along with the spiral valve in later stages.

Mayr ('97) in his account of the development of the pancreas in Pristiturus and Torpedo incidentally describes the condition of the liver in several embryos. His description coincides with that of Balfour and Brachet, but he emphasizes the fact that in early stages the pars hepatica is single anteriorly and that the two lateral pouches diverge from the median line as they extend backward.

Choronschitzky ('00) published a paper of some magnitude, describing the development of the liver and certain other viscera in all classes of vertebrates. Torpedo was employed as a representative of the fishes. He gave an account of four stages of this form, the youngest being one in which the liver consisted of a median and two lateral pouches from which four 'secondary' pouches sprang and the oldest one in which the liver had reached a length of 1.1 mm. as measured from transverse sections and was a solid parenchymous organ. He traced the separation of the liver from the gut and the formation of the gall bladder in some detail and followed out in a general way the development of the two larger hepatic ducts. On the whole his description is a confirmation of that of Brachet. The reconstruction method was apparently not employed.

The work of Debeyre ('09) is primarily a study of the origin of the hepatic cylinders. However, working on Laguesse's Acanthias material, he confirms that author's account of the early stages of the liver, including the location of the gall-bladder anlage in the extreme posterior end of the hepatic diverticulum.

The papers of Braus ('96), Holm ('97), and Minot ('00) deal mainly with the histogenesis and vascularization of the liver, but contain incidental references to its early development. Braus and Minot accept Balfour's account as essentially correct.

III. DEVELOPMENT OF THE LIVER IN EMBRYOS FROM 3 TO 10 MM. IN LENGTH

In Acanthias the first evidences of liver development are to be seen in embryos of 19 to 21 segments. At this stage, which is a little younger than No. 16 of the Normal plate series and lies between Balfour's stages G and H, the embryo shows a distinct head bend and the medullary canal is closed except for the large neuropore. The archenteron which is still in quite a primitive condition is outlined in figure 1, a graphic reconstruction from transverse sections. The fore gut is separated from the entoderm of the blastodisc and is approximately one-sixth the length of the body. The lateral walls of the mid and hind gut are still separated at their bases almost throughout by a considerable ventral cleft, and meet only a few sections anterior to the tail to form the lower and hinder walls of the neurenteric canal. The first gill pouch is a shallow depression, present on one side only. The second gill pouch is indicated by a very slight depression in the dorsal part of the lateral wall of the pharynx immediately posterior to the first pouch. Its ventral portion is not as yet evaginated.

As the fore gut approaches its connection with the blastodermic entoderm it is at first broadly ovoid in cross section with the narrower end of the oval upward and its vertical diameter increases posteriorly. Back of the fore gut the archenteron is flattened transversely until it is little more than a high, narrow fold of entoderm, the transverse diameter of which is less than one-fourth of the vertical diameter. The lateral walls of the gut for a short distance behind and also a little in front of the point of union of the fore gut and the mid gut are differentiated into dorsal and ventral zones. The epithelium of the dorsal zone is from 25 to 30 μ in thickness and contains two or more rows of more or less interlocking oval nuclei. This is the primitive condition found throughout the walls of the archenteron, both dorsally and ventrally in earlier stages. Close to the lower border of the dorsal zone there is on either side a shallow and not always continuous longitudinal groove which later becomes a definite and important landmark. For these I suggest the name para-archenteric grooves. The ventral zone in this region, as is seen from following its later history, represents the liver anlage. Here the epithelium is approximately half as thick as that of the dorsal zone, and the nuclei, which form a single row only, lie in the basal portion of the epithelium. Ordinary stains do not bring out definite cell walls in either the dorsal or ventral zones at this stage. There

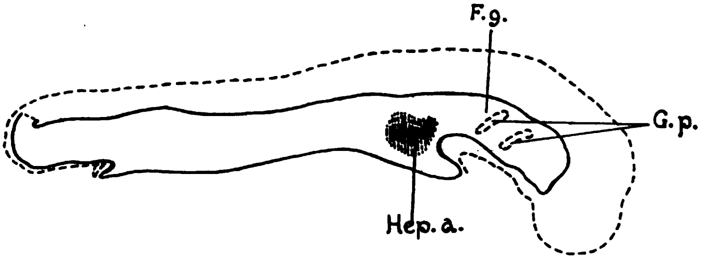
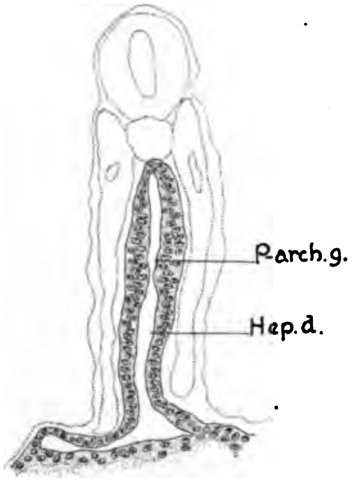


Fig. 1 Lateral view of the archenteron of an embryo of 19 to 20 segments, 4.0 mm. in length (H.E.C. 930). × 20. *F.g.*, fore gut; *G.p.*, gill pouches; *Hep.a.*, hepatic area.

are no definite boundaries, except the dorsal one to the hepatic area at this time. The arrangement of nuclei just described extends ventrally nearly to the point where the lateral walls of the archenteron turn laterad as a part of the blastoderm. Longitudinally the hepatic region extends forward a little past the posterior end of the fore gut to become indistinguishable in the general ventral enlargement of the pharynx already referred to. Its characteristics are less noticeable as we follow the gut posteriorly and 100 μ behind the point of union of fore and mid gut the hepatic area is indistinguishable from the other entoderm. There is but a slight indication of evagination of the liver area. Although the lumen between the walls of the ventral zone is nearly twice as wide as that above, this width is due mainly to the decrease in the thickness of the walls themselves, the entire transverse diameter of the gut being a little greater dorsally than ventrally.

A slightly older embryo having 24 trunk segments and 3.6 mm. in length, which is a little more advanced than the Normal plate No. 20 (Scammon '11), gives a clearer picture of the liver anlage. The pharynx, from which two well formed gill pouches project and fuse with the skin ectoderm, is followed by a short segment of gut which represents both the oesophagus and the anterior part of the stomach. This segment is elongately oval in cross section with very much thickened lateral walls. It is somewhat produced ventrally as it approaches the anterior wall of the yolk-stalk, and in this ventral region shows lateral expansion. The archenteron extends forward forming a large anterior recess above the yolk in front of the anterior wall of the yolk-stalk. Immediately behind the point of union of the fore gut with the yolk-stalk the archenteron has the same form as in the younger embryo, being greatly elevated and flattened transversely. The distinction between dorsal and ventral zones is fairly marked, and the para-archenteric grooves can be traced along the gut above the hepatic region, although in places they are very faint and shallow. The ventral zone in this region is now distinctly curved outward, forming a pair of shallow, lateral diverticula which extend approximately 100 μ posterior to the anterior vitellointestinal junction. The walls of these lateral diverticula are of the same thickness as those of the dorsal zone, i.e., 25 to 30 μ, but the nuclei are somewhat more elongated and lie at the basal ends of the cells, leaving a clear zone towards the lumen of the gut. The boundaries of the cells are faintly distinguishable.



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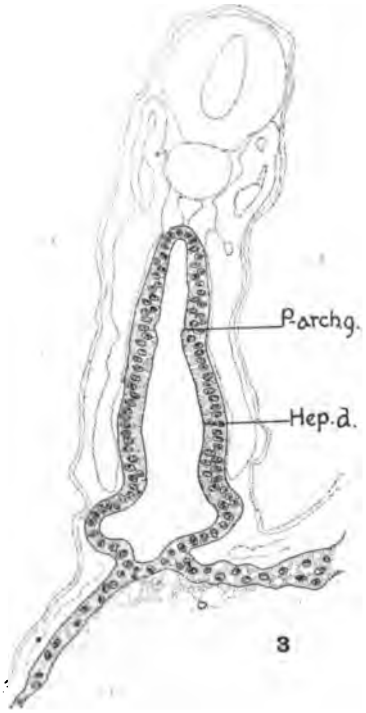

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Fig. 2 Transverse section of an embryo of 19 to 20 segments, 3.0 mm. in length (K.U.E.C. 451). 0.06 mm. posterior to the anterior wall of the yolk stalk. × 100. *Hep.d.*, hepatic diverticulum; *P-arch.g.*, para-archenteric groove.

Fig. 3 Tranverse section of an embryo of twenty-four segments, 3.6 mm. in length (S.C. 14). 0.09 mm. posterior to the anterior wall of the yolk stalk. × 100. *Hep.d.*, hepatic diverticulum; *P-arch.g.*, para-archenteric groove.

Above and below the diverticula the nuclei are broadly oval and scattered through the thickness of the epithelium, and there are no distinct cell outlines to be seen with ordinary stains. Figure 3 is a transverse section of this embryo 90 μ behind the anterior wall of the yolk-stalk.

A distinct step in development is shown by an embryo 5 mm. in length (K.U.E.C. 449[3]). This embryo has segments and three gill pouches, none of which open to the exterior. The gut is still connected with the yolk entoderm by a yolk-stalk, which has an antero-posterior diameter equal to nearly half the length of the archenteron. A lateral view of the liver region is outlined in figure 4, which is a graphic reconstruction, with the hepatic area outlined with broken lines. The lateral hepatic diverticula are somewhat better defined, both dorsally and ventrally, than in the preceding stage and are somewhat deeper. They show in a more marked way the histologic differentiation described for the preceding embryo, as is illustrated by figures 5 and 6, for the cells have lengthened so rapidly that epithelium is one-fourth to one-half thicker than that of the dorsal zone of the gut above it. Numerous mitoses indicate the rapid growth now taking place in this region. It will be noticed from figure 4 that the lateral liver diverticula now extend forward distinctly beyond the anterior wall of the yolk-stalk. Here the right and left pouches are fused, forming a single median and ventral pouch in the posterior part of the fore gut. The finer structure of this anterior part is illustrated in figure 5. It shows the same character and distinction from the remainder of the gut wall as does the posterior part of the anlage. The right and left omphalo-mesenteric veins are now present, although of small caliber.

A slightly older embryo 6.4 mm. long shows somewhat the same stage of development and the liver region has been reconstructed in wax. Figures 30 and 31 show anterior and right lateral views of this object. The entire embryo is inclined markedly to the left. The fore gut, ovoid in cross section, becomes immediately flattened and triangular after passing the anterior wall of the yolk-stalk. The lateral hepatic diverticula are distinctly outlined and fuse together, forming anteriorly a ventral pouch in the floor of the fore gut. The antero-posterior length of the hepatic anlage

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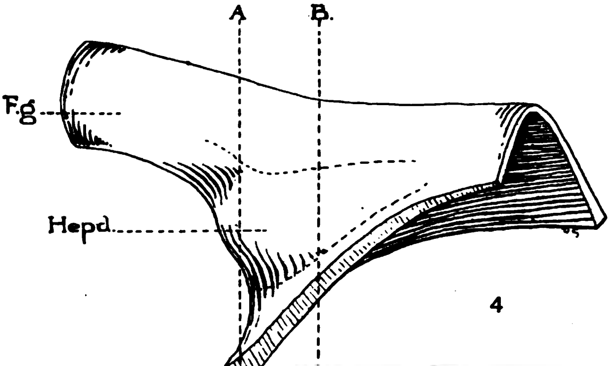
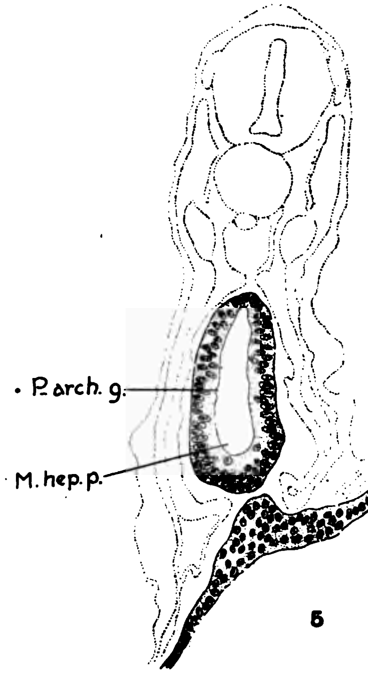
[3] The following designations of embryos are employed in this paper: H.E.C. = Harvard Embryological Collections. K.U.E.C. = Kansas University Embryological Collection. S.C. = Author's Collection.





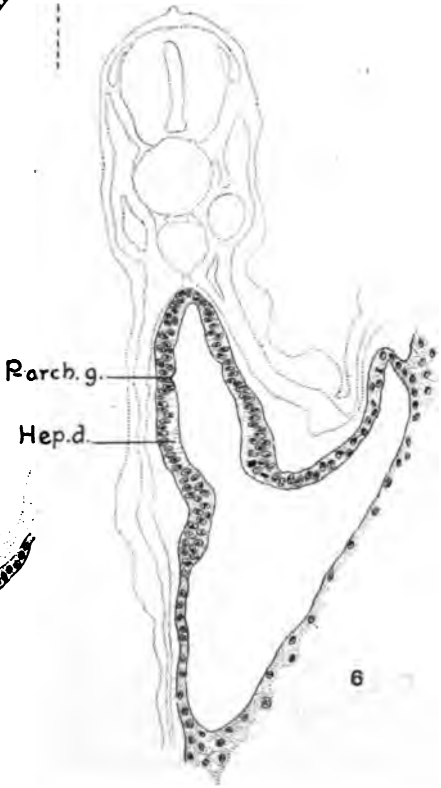


Fig. 4 Graphic reconstruction of a portion of the fore and mid gut of an embryo 5 mm. long (K.U.E.C. 449). × 50. The dotted lines indicate the borders of the hepatic thickening. Lines *A* and *B* indicate the planes of sections represented in figures 5 and 6. *F.g.*, fore gut; *Hep.d.*, hepatic diverticula.

Fig. 5 Transverse section of the same embryo 0.03 mm. in front of the anterior wall of the yolk stalk. × 100.

Fig. 6 Transverse section of the same embryo 0.06 mm. posterior to the anterior wall of the yolk stalk. × 100. *Hep.d.*, hepatic diverticula, *M.hep.p.*, anterio-median hepatic pouch, formed by the fusion of the lateral hepatic diverticula. *P-arch.g.*, para-archenteric grooves.

is 0.30 mm. The length of the fused anterior parts of the lateral diverticula is 0.13 mm., while that of the still separated posterior portions is approximately 0.17 mm. The posterior part of the lateral liver anlagen, however, can hardly be called diverticula, as they are little more than thickened plates of cells. The histologic differences between the liver diverticula and the remainder of the archenteron walls are shown in figure 7. The nuclei in the walls of the lateral diverticula are no longer arranged in a single layer, but are irregularly placed in the basal halves of the cells. Their elongation is noticeable. The cytoplasm of the cells of the hepatic area is condensed and stains darkly as compared with that of the cells above. The para-archenteric grooves are very

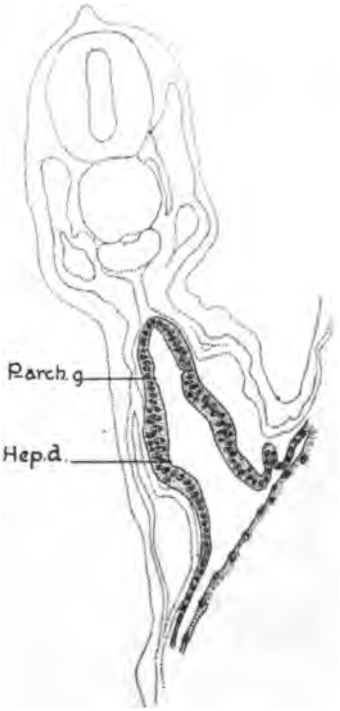


Fig. 7 Transverse section of an Acanthias embryo 6.4 mm. long (S.C. 19), 0.05 mm. posterior to the anterior wall of the yolk stalk. × 100. *Hep.d.*, hepatic diverticula; *P-arch.g.*, para-archenteric groove.

broad and shallow and can be followed with difficulty above the lateral diverticula.

Two marked changes now take place in the liver anlage, bringing about the condition described by Brachet ('96) and others as the primitive one. These are the appearance of the gall bladder and the distinct lateral and dorsal evagination of a part of the lateral hepatic diverticula. An embryo 7 mm. in length (H.E.C. 752), which is probably a little younger than No. 21 of the Normal plate series and a little older than Balfour's Stage H is the earliest specimen in which I have observed any indication of the gall bladder. Both the separated posterior parts and the anterior median pouch, formed by the fusion of the anterior ends of the lateral hepatic diverticula, are more pronounced than in the embryo just described. The anterior wall of the yolk-stalk is much thickened over its entire extent, but particularly just below the point where it becomes continuous with the floor of the fore gut. The gall bladder is represented by a very shallow median depression at this place. Numerous mitoses indicate that the epithelium is growing rapidly in this region. The early evagination of the gall bladder must take place with some rapidity, as it is very difficult to find any specimens between the stages when this structure is entirely absent and when it is a deep, well-marked pouch.

A specimen which illustrates both the early development of the gall bladder and the growth of the lateral diverticula, and is quite comparable with the first members of Hammar's ('93) and of Brachet's ('96) series of models, has been reconstructed and figures 33 and 34 are anterior and left lateral views of the model. This specimen, which is 7.5 mm. in length (H.E.C. 1503 and No. 24 of the Normal plate series), has 54 to 55 trunk segments and four gill pouches, two of which open exteriorly. The spiral valve makes one and one-third turns of the gut. The distance from the last (fourth) gill pouch to the anterior wall of the yolk-stalk is approximately one-fourth of the complete length of the alimentary canal and about equal to the antero-posterior diameter of the yolk-stalk. The lateral hepatic diverticula are differentiated into three parts. Anteriorly they are fused, forming the single median pouch already mentioned which now projects downward from the floor of the fore gut. Continuous with the median pouch thus formed are the middle parts of the diverticula which are expanded laterally and dorsally and which will be referred to hereafter as the lateral hepatic pouches. The lateral hepatic pouches extend backward as far as the anterior wall of the yolk-stalk, and there become continuous with the posterior parts of the lateral diverticula which remain almost unchanged from their slightly expanded condition of earlier stages, and which will be referred to as the pars ductus, as it is from them that the major portion of the ductus choledochus is formed. The left lateral pouch becomes continuous with the pars ductus of that side without any distinct line of demarcation. On the right side, however, the pouch ends abruptly by projecting nearly at right angles from the gut wall. Deep grooves, of which the left is the more pronounced, intervene between the latter walls of the ventral part of the fore gut and the mesial walls of the dorsally growing lateral pouches. These indicate the beginning of the process by which the liver will be eventually cut off from the gut tube above. The gall bladder is present as a deep ventral pouch, lying between the median liver pouch in front and the anterior wall of the yolk-stalk behind. Its walls are directly continuous with the ventral part of the liver pouch and the pars ductus above, but a slight longitudinal groove marks the boundary between the structures. This groove becomes deeper as it proceeds anteriorly until a point is reached about one-fifth of the length of the pouch from its anterior wall. Here the groove is entirely absent and there is thus left a small anterior expanded segment of the gall bladder stalk which is the anlage of the primitive cystic duct. A point worth emphasis is that the entire liver anlage shows a slight rotation to the left and that the left lateral liver pouch shows a greater dorsal growth than does the right. The para-archenteric grooves have remained unchanged.

The changes which now follow are those of passive growth. The lateral pouches expand transversely and become almost globose in outline. At the same time there is a slight growth dorsally which deepens the groove between them and the gut mesially. The pars ductus expands somewhat and becomes more sharply marked off from the archenteron posteriorly. The anterior median pouch also shares in this expansion but shows no other changes. Likewise the gall bladder becomes rotund, a distinct groove intervenes between its dorsal-anterior angle and the liver anlage and a ventral notch of some depth separates the sack from the anterior wall of the yolk-stalk behind. In front of this ventral notch the sac is still continuous with the liver anlage proper, but the longitudinal construction between the two structures mentioned in the description of the preceding embryo is present in a more distinct form. These changes are illustrated in figures 35 and 36, of a wax reconstruction from an embryo 9 mm. in length, the general anatomy of which has been previously illustrated in graphic reconstruction in figure 11 of the Normal plates of Acanthias.

A little later, as shown in an embryo of sixty somites with three open and two closed gill pouches and two complete turns of the spiral valve, the lateral pouches lose their expanded outline, and becoming flattened laterally, enter upon a decided dorsal growth (figs. 37 and 38). At the same time their posterior margins become sharply differentiated so that they extend out from the gut at an abrupt angle and their distal edges show several slight irregularities. The anterior median pouch remains practically unchanged. The lateral grooves along which the liver eventually separates from the fore gut above it are now considerably deepened and extends the entire length of the line of attachment of the liver evagination, although they are still shallow anteriorly. The left lateral pouch bears on its lateral surface three small longitudinal ridges. These together with the dorsal irregularities mentioned above constitute the anlagen of hepatic tubules and will be discussed in the section dealing with these structures. The gall bladder while no larger than in the preceding embryo is separated from the anterior wall of the yolk-stalk by a deeper ventral notch and the constriction between the sack and the median part of the liver above is more pronounced. Both this and the preceding stage show a slight but distinct rotation of the anterior part of the liver to the left around the fore gut as an axis.

A considerable advance in development is seen in an embryo only a millimeter longer than the preceding one. This specimen corresponds fairly well with Balfour's stage I, or No. 24 of the Normal plate series the embryos of which measured 11.5 mm. It has sixty-five segments, three open gill slits and two unopened gill pouches and four turns of the spiral valve. A reconstruction of the liver and adjoining archenteron is illustrated in figures 28, 39 and 40. The process by which the hepatic pouch will eventually be separated from the gut above is well under way. The median hepatic pouch from which the lateral pouches spring and to which the lateral pouches are attached is rather triangular in cross section anterior to the origin of the lateral pouches. The broader part is below and the narrow dorsal extremity joins the floor of the fore gut anteriorly a little to the right of the median line (fig. 8 *A*).

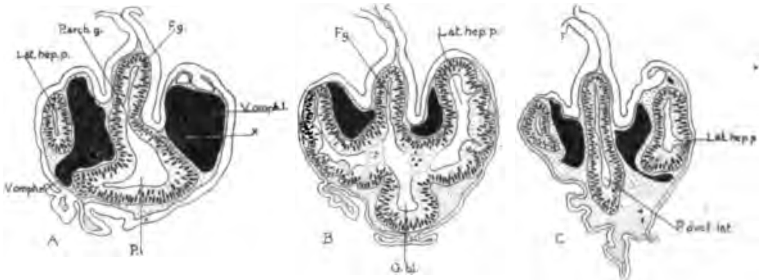


Fig. 8 Three transverse sections through the liver region of an Acanthias embryo 10 mm. long (S.C. 20). × 50. *A*, Through the anterior part of the median hepatic pouch (pars hepatica mediana). *B*, Through the posterior part of the median pouch (pars ductus mediana). *C*, Through the gut just posterior to the liver proper showing the pars ductus lateralis. *F.g.*, fore gut; *G.bl.*, gall bladder; *Lat.hep.p.*, lateral hepatic pouch; *P-arch.g.*, para-archenteric groove; *P.duct.lat.*, pars ductus lateralis; *P.*, pars hepatica mediana; *V.omph.l.*, *V.omph.r.*, left and right omphalo-mesenteric veins; *X*, anlage of the ductus choledochus.

The ventral part of the gut is also rotated to the right so that these two structures join at an oblique angle thus forming a broad shallow groove on the left hand side while on the right they form a smooth somewhat convex surface. Posteriorly the median hepatic pouch is continuous with a short segment of the gut which in turn becomes attached to the yolk stalk. The para-archenteric grooves are still distinguishable and mark the plane of union between the hepatic anlage and the gut (fig. 8, *A*, *B* and *C*,-*P-arch. g.*). Two parts can be distinguished in the median pouch, an anterior one which projects a little in front of the anterior end of the attachment of the lateral pouches, and a posterior part which is directly continuous with the former and from which the lateral pouches and the gall bladder take origin. Although these divisions are not sharply marked off at present, they later become quite distinct. The anterior one from its later history may be called the pars hepatica mediana because it shares with the lateral pouches or pars hepatica lateralis in the formation of hepatic ducts and trabeculae. The posterior later develops into a part of the ductus choledochus and may be called the pars ductus mediana in distinction to the anterior and to the pars ductus lateralis formed from the posterior portions of the original hepatic diverticula. The upper surface of the posterior part or pars ductus mediana of the median pouch lying on either side of this dorsal connection with the gut already shows a peculiar modeling indicative of the course which will be eventually taken by the ductus choledochus (fig. 28). There is a marked expansion which extends from the right anterior angle of the median pouch obliquely backward to the posterior left corner. The anterior, i.e., right portion of this swelling is the more marked. From the posterior edge of the middle pouch this expansion is continued backward into the mid gut as a symmetrical lateral expansion of the ventral part of the 'connecting piece' between the hepatic and stomach anlagen above it to the yolk-stalk and overlying gut. As seen from their position these lateral expansions are the remains of the posterior ends of lateral hepatic diverticula or pars ductus lateralis (fig. 8, *C*). Thus there can already be recognized two distinct parts of the ductus choledochus: an anterior asymmetric portion and a posterior part which is symmetrically placed.

The lateral pouches have continued their dorsal growth and now extend as far upward as the dorsal surface of the gut. Their upper parts are expanded, particularly posteriorly, so that a proximal constricted stalk and a distal expanded portion can be distinguished. In earlier stages as shown by figures 33 and 37, the origin of each lateral pouch was continuous with the entire lateral edge of the median one, but at this stage it is confined to the posterior four-fifths of this edge. The dorsal part of each lateral pouch is curved a little medially. All the external lateral surface of each lateral pouch is corrugated with rather irregular longitudinal ridges which are somewhat broken by shallow transverse fissures (fig. 40). Similar ridges are forming on the ventral surface of the median pouch anterior to the gall bladder. The dorsal edge of each median pouch is also rendered exceedingly irregular by the several small pouches springing from it. All of these structures are the anlagen of hepatic tubules the formation of which was referred to in the description of the preceding embryo.

The gall bladder is now a large thick-walled sac, ovoid in shape and somewhat flattened dorso-ventrally. Distinct grooves separate it from the median liver pouch and connecting piece behind. and along its dorsal edge. These grooves are however deeper posteriorly than anteriorly as might be expected from their history in earlier stages. The extreme anterior tip of the gall bladder is drawn to a point and projects very slightly forward below the anterior part of the median liver pouch.

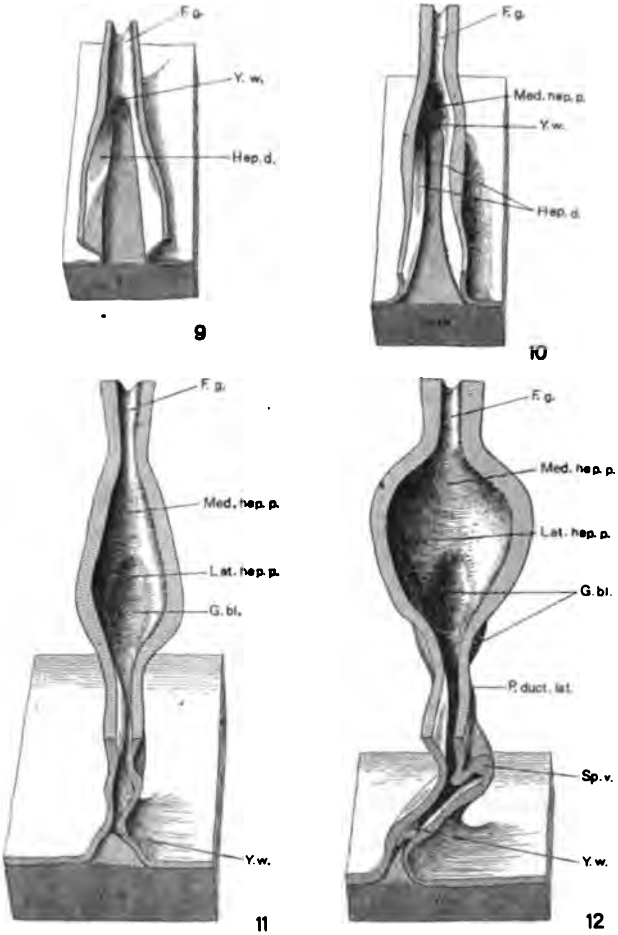
IV. CONCLUSIONS

All the main divisions of the liver are now established and before giving an account of their later history it may be well to summarize the development of the organ up to this stage. The semidiagrammatic models shown in figures 9 to 12, illustrate this process. They are of embryos 3.6, 6.4, 7.5 and 9 mm. long respectively, and are based upon wax reconstructions and measurements of specimens described in the preceding pages. In each a portion of the mid and fore gut is represented as resting on a block of yolk. The dorsal half of the archenteron is cut away so that one looks down on the interior of the ventral half of the gut from above and a little behind. Figure 9 shows the liver as a pair of shallow lateral diverticula, lying mainly behind the point of union of fore gut and blastodermic entoderm or the anterior wall of the yolk-stalk. As the embryo is elevated and farther separated from the blastoderm the anterior wall of the yolk-stalk retreats posteriorly. This brings the anterior ends of the lateral diverticula in contact and they fuse more and more, forming the median liver pouch and producing the condition shown in figure 10.

Here the liver anlage is *U*-shaped with the limbs of the *U* turned posteriorly and slightly divergent. The fusion of the lateral diverticula is continued along with the posterior progression of the anterior wall of the yolk-stalk. At the same time parts of these structures undergo unequal growth. In each diverticulum the middle part above and a little behind the fused median portion begins a rapid dorsal and lateral growth, producing the structures known variously as 'lateral pouches' 'ébauche hépatique,' and 'Seitendivertikel.' The posterior parts of the primitive lateral diverticula, or pars ductus, which extend backward to or beyond the anterior wall of the yolk-stalk share but little in this growth, but remain unchanged in their primitive condition as a pair of shallow lateral diverticula until at a much later period, they are transformed into a part of the ductus choledochus.

In the meantime the gall bladder arises as an out-pouching of the dorsal part of the anterior wall of the yolk-stalk and being somewhat cut off from that structure by the posterior growth of the fore gut comes to lie between it and the median liver pouch anteriorly and with the lateral median pouches bounding its sides. This stage is represented in figure 11. Figure 12 shows a somewhat later stage modified from the preceding by the greater expansion of all parts of the hepatic structure and by a still greater elongation of the fore gut.

This account of the development of the liver is to some extent in accord with that given by Hammar ('93) as opposed to the idea of a single median ventral anlage as advanced by Balfour ('76), Laguesse ('93), Brachet ('96) and Choronschitzky ('00).



Figs. 9, 10, 11, 12 A series of semi-schematic reconstructions to illustrate the early development of the liver; all × 50. The plan of reconstruction is explained in the text on p. 350. *F.g.*, fore gut; *G.bl.*, gall bladder; *Lat.hep.p.*, lateral hepatic pouches; *Med.hep.p.*, median hepatic pouch; *Hep.d.*, hepatic diverticula; *P.duct. lat.*, pars ductus lateralis; *Sp.v.*, spiral valve; *Y.w.*, anterior wall of the yolk stalk.

Fig. 9 Embryo 3.6 mm. long.
Fig. 10 Embryo 6.4 mm. long.
Fig. 11 Embryo 9 mm. long.

From a short study of early Torpedo embryos I am inclined to think that in this form the stage in which the liver exists as a pair of lateral diverticula must be very brief if at all present, because embryos of this form separate from the blastoderm at an earlier period than do Acanthias embryos and have a comparatively small yolk-stalk. The rapid formation and elongation of the fore gut accompanying these changes may involve the hepatic areas before they are differentiated as pouches. As regards Acanthias and probably other Selachii, it appears to me very probable that investigators have been misled from a study of embryos which have advanced to a considerable extent in the process of development. It is interesting to note that in embryos of other groups of animals possessing large yolk-laden ova the liver forms at a stage when other organs are in quite a primitive condition, and in only slightly teleolecithal ova the time of origin is still younger. At the time when Balfour, Brachet and Laguesse record the appearance of the liver in elasmobranch fishes the embryo is well established, several gill slits are fully formed, the sense organs are completely invaginated, the spinal nerve anlagen are laid down and the limb fundaments are about to appear. The earliest anlage of the liver is not extensive and can hardly be recognized without a previous study of somewhat later stages. Again after the primitive paired liver diverticula are well formed they are often to a considerable extent obliterated by the falling of the embryo to one or the other side as it becomes top-heavy by separation from the yolk which has supported it up to this time. This flexure causes one or more large transitory folds which tend to render inconspicuous the pouch in the side upon which the embryo comes to lie, and at the same time almost obliterates the opposite pouch by stretching. The histologic characters of these areas remain unchanged however. Figure 13 is a cross section through the liver region of an embryo 4.8 mm. in length, showing these changes.

Although in this form of selachian at least the anlage of the liver is a paired one, it does not follow that this is the original condition of the structure. It seems probable that it has been brought about purely by the mechanical influence of the large amount of yolk present by which the original tube of entoderm is spread out plate-like upon an almost flat surface of the yolk substance. In such a case the ventral portion of the original tube would form the peripheral portion of each lateral half of the plate and the folding of the plate into a tube again in the course of the separation of the embryo from the yolk would approximate once more the two separated parts. These lateral diverticula may then be regarded simply as the potential halves of an original ventral pouch which begin their expansion before their union along the ventral median line takes place. As might be expected

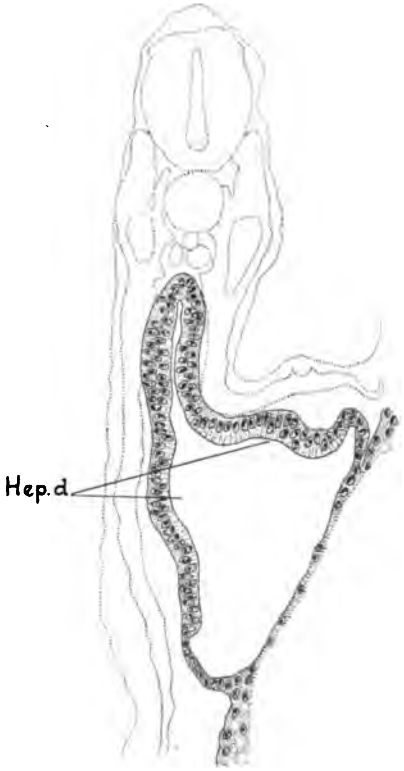


Fig.13 Transverse section of an Acanthias embryo 4.8 mm. long (H.E.C. 1398), showing the effect of the inclination of the embryo upon the lateral hepatic diverticula. × 50. *Hep.d.*, hepatic diverticula.

this union does not occur at the same time along the entire antero-posterior length of the liver but proceeds from the cephalic end backward, thus producing the *U*-shaped figure shown in figures 9 and 10.

It appears that too much importance has been placed upon the position of the liver anlagen in regard to the anterior intestinal portal. Laguesse ('93), Brachet ('96) and Choronschitzky ('00) all emphasize this point. This structure, however, is constantly shifting posteriorly as has been demonstrated by Mayr ('97), and the location of the liver in front of it holds good only for stages which are well advanced. The location of the liver as immediately behind the sinus venosus is perhaps of more value, but in Acanthias at least, the liver is present before the two omphalomesenteric veins become confluent to form the endothelial heart, or indeed before they are represented by definite endothelial tubes.

Laguesse has already called attention to the fact that in Acanthias the gall bladder appears somewhat later than the remainder of the hepatic apparatus, and seems to be developed from the anterior wall of the yolk-stalk rather than from the posterior part of the median liver pouch. Hammar ('93, '97) appears to hold the same opinion in regard to Torpedo. I believe that my sections and models bear out this conclusion and that this structure can be properly interpreted as an organ arising quite separate from the hepatic anlage at the juncture of the pars ductus of the lateral liver diverticula and the floor of the gut, as represented by the anterior wall of the yolk-stalk portal. The shifting of the sac anteriorly so that its duct comes to lie in front of the openings of the hepatic ducts into the ductus choledochus will be discussed in the following section.

All specimens after the stage when the median and lateral hepatic pouches are formed show a small but constant rotation of the hepatic anlage to the right. This rotation seems without doubt to be a part of that greater one which produces the spiral valve. Like the latter it is from the left to the right side, i.e., clockwise around an axis corresponding to the longitudinal axis of the gut, and it is coincident with it, appearing when the embryo has from 50 to 60 segments and has reached a length of 6 to 7 mm.

Its extent is but slight as compared with that of the posterior portion of the gut, being at most not over 15 degrees. The hinder and lower portion of the hepatic anlage is less affected by this twisting than is the anterior free part presumably because its attachment to the vitelline duct is still considerable in extent and must offer some resistance to whatever force it may be that produces the rotation. That this portion of the alimentary tract is affected to a slight degree however is shown by the broad shallow groove which appears in the posterior half of the left wall of the yolk-stalk and which has been figured and described in the Normal plates (Scammon '11), under the term 'Lateral groove of the vitelline duct,' and which may be seen in the figures of reconstructions of embryos 7.5, 9 and 11.5 mm. in length respectively, in that paper. The gut anterior to the yolk-stalk also shows some rotation, being twisted to the right as is indicated by the angle formed by its lumen with the mid-sagittal plane of the body.

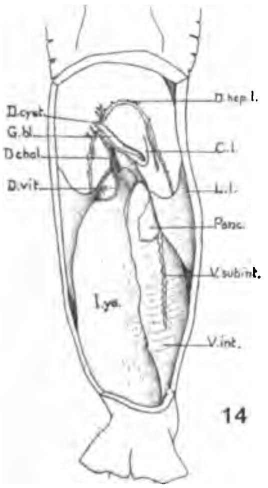
PART II

I. DESCRIPTION OF FULLY FORMED BILIARY APPARATUS

Before attempting to describe the development of the gall bladder and liver ducts, it may be well to outline the form of these structures in the late embryo or new-born fish and to present the terminology which will be employed in the remainder of this paper.

In large embryos and new-born specimens of Acanthias the liver is a large viscus occupying nearly half of the abdominal cavity. It consists of two lateral lobes which are united anteriorly by a median mass which stretches completely across the body cavity posterior to the septum transversum. From the right ventral and posterior margin of the median mass a small pointed process extends backward and to the left. As the gall bladder is imbedded in this mass it has been termed the cystic lobe. The cystic lobe lies directly ventral to the stomach and to the left of the cephalic end of the large internal yolk sac.

The gall bladder is an elongated tubular sac which lies along the right margin of the cystic lobe. It is imbedded in hepatic parenchyma except for a little of the right surface which receives a peritoneal investment. The cystic duct arises from the anterior end of the gall bladder and proceeds directly dorsally. It then makes a sharp semicircular curve and proceeds posteriorly to join the ductus choledochus. In older embryos and adults there is no line of demarcation between the cystic and common bile ducts. The ductus choledochus extends backward ventral to the



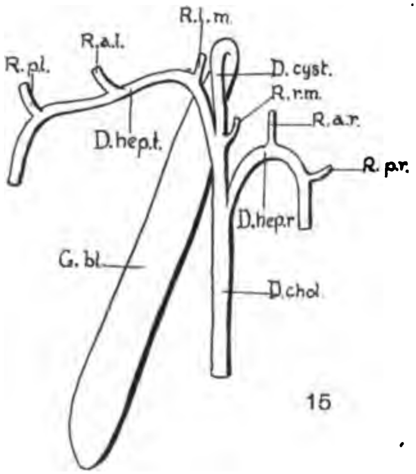


Fig. 14 A dissection of an Acanthias embryo 20 cm. in length. × ¾. The ventral abdominal wall has been cut away and the vitelline duct severed at its connection with the internal yolk stalk. The gall bladder and main hepatic ducts have been dissected out. The large veins of the liver have been omitted from this drawing. *C.l.*, cystic lobe; *D.chol.*, ductus choledochus; *D.cyst.*, cystic duct; *D.hep.l.*, left hepatic duct; *D.vit.*, vitelline duct; *G.bl.*, gall bladder; *I.y.s.*, internal yolk sac; *L.l.*, lateral lobe; *Panc.*, pancreas; *V.int.*, valvular intestine; *V. subint.*, subintestinal vein.

Fig. 15 Diagrammatic representation of the gall bladder and liver ducts of Acanthias as seen from above. *D.chol.*, ductus choledochus; *D.cyst.*, cystic duct; *D.hep.l.*, left hepatic duct; *D.hep.r.*, right hepatic duct; *G.bl.*, gall bladder; *R.a.l.*, anterior left hepatic ramus; *R.a.r.*, anterior right hepatic ramus; *R.l.m.*, left medial hepatic ramus; *R.p.l.*, posterior left hepatic ramus; *R.p.r.*, posterior right hepatic ramus; *R.r.m.*, right medial hepatic ramus.

internal yolk sac joining the valvular intestine on the left side of the first turn of the spiral valve.

Figure 15 shows the gall bladder and hepatic ducts in diagram. The main right and left hepatic ducts join with the ductus choledochus obliquely, the left gaining entrance in front of the right. The distance between the ostia of the two ducts varies in different specimens. The left duct after extending a short distance anteriorly arches far out laterally and there turning backward passes posteriorly in the left lateral lobe. The right duct makes a sharp arch anteriorly and then passes backward into the right lobe.

The main lateral hepatic ducts give rise to numerous small hepatic tubules and to several larger rami. The former are extremely irregular in form, origin and number, but the latter, although displaying great variation in position can in most cases be reduced to the following classification: (1) right medial hepatic ramus, (2) left medial hepatic ramus, (3) anterior right hepatic ramus, (4) posterior right dorsal hepatic ramus, (5) anterior left hepatic ramus, (6) posterior left dorsal hepatic ramus.

The right medial hepatic ramus varies considerably in the place of origin, commonly it is attached to the right duct near its proximal end. The left medial hepatic ramus commonly takes origin from the proximal part of the left hepatic duct but may in some cases be attached to the ductus choledochus or even to the base of the right hepatic duct. The anterior right and the anterior left rami generally arise from the summit of the anterior arch formed by each of the main hepatic ducts, but the left ramus may attach either to the ductus choledochus, or as I have observed in one embryo, to the base of the main right hepatic duct. The posterior dorsal hepatic rami arise from the hepatic ducts either at the lateral extremity of the anterior arch or in the anterior part of their posterior course. They seem to be fairly constant in position. Minor variants from the above scheme are common and simple rami differ much in size or may be replaced by two or more smaller ones. The terminology used here is based upon the development of these structures as will now be described.

II. DEVELOPMENT OF THE HEPATIC DUCTS AND THEIR RAMI

The elements entering into the formation of the hepatic ducts are the anterior portion of the median liver pouch or pars hepatica mediana, and the right and left lateral hepatic pouches or the pars hepatica lateralis.[4] These structures are converted into the main hepatic ducts found in the fully developed embryo by means of reduction in caliber both relative and actual, by elongation, and by partial separation from the posterior portion of the median liver pouch or pars ductus mediana. That such processes take place has been recognized by Balfour ('76), Hammar ('93), Brachet ('96), and other investigators. The details have not been described. The minor ducts are formed in Acanthias in much the same manner as are the major ones, by the differentiation and elongation of the proximal parts or pedicles of certain fairly definitely placed groups of tubules which arise from the surface of the embryonic structures which form the main ducts. This method of formation of the minor ducts probably holds only for selachians in which the omphalo-mesenteric veins are comparatively small and develop at a late stage.

An account of the development of these ducts may begin with the description of an embryo 15 mm. in length (H.E.C. 227 and No. 26 of the Normal plate series) the general anatomy of which is illustrated in figure 13 of the Normal plates of Squalus acanthias.

The main divisions of the liver of this specimen and the proximal part of the hepatic tubules arising from them have been reconstructed and figures 41, 42 and 44 are right lateral, left lateral and anterior views of this object.

The median hepatic pouch is completely separated from the gut above and this separation has extended backward converting the pars ductus lateralis into a short tube of large caliber. This is the middle part of the ductus choledochus. It joins with the floor of the duodenum a little to the right, thus preserving the same relation observed in younger embryos. The obliquely placed swelling upon the dorsal surface of the median pouch which represents the course of the distal portion of the ductus choledochus is present but is not so marked as in the embryo 10 mm. in length described in the preceding section (p. 348).

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[4] The use of this and the following terms of this paragraph is somewhat of a departure from the classification of the components of the selachian liver pouch into 'pars cystica' and 'pars hepatica' as proposed by Brachet ('96, '97) on the basis of a similar classification employed by Goeppert ('93) in his description of the development of the liver in Teleosts. The term 'pars cystica' as used by Brachet includes the 'pars ductus mediana' and 'pars ductus lateralis' as employed here, as well as the anlage of the gall-bladder and cystic duct. If the conception as presented here, of the gall bladder as an organ with an origin distinct from the liver, is a correct one, then this term 'pars cystica' can hardly be properly applied

The pars hepatica mediana or anterior part of the median pouch is broadly continuous with the pars ductus medialis behind and with the lateral pouches posteriorly and laterally. Its anterior surface (fig. 44) is rendered extremely irregular by the formation of a number of hepatic tubules. The origin of these structures from ridges in the pars hepatica was noted in connection with the description of an embryo 10 mm. in length, in the preceding section of this paper. At the present stage the tubules arising from the pars hepatica mediana are little more than short conical evaginations of the pouch wall and only one shows any evidence of the complex branching which all soon undergo. The tubules of the pars hepatica mediana are divided into two groups, a right and a left, by a deep vertical furrow which lies somewhat to the left of the median plane and extends from the dorsal to the ventral surface of the pouch. The right subdivision thus formed is more extensive than the left, but a smaller number of tubules arise from it. The groups of tubules established by this subdivision will be termed in this paper the right medial group and the left medial group respectively. The lower surface of the pars mediana remains smooth at this stage and rests upon a mass of mesenchyma which extends from the anterior surface of the gall bladder to the anterior mesothelial wall of the liver.

to all of these structures and to use it for structures which are later wholly incorporated in the ductus choledochus and not in the vessica fellae or its duct at all, seems inadvisable. The use of the expression 'mediana' in connection with 'pars ductus' is not intended to convey the meaning that this portion of the ductus choledochus is a direct derivative of the median part of the gut primarily, but that it is formed from the median pouch produced by the fusion of the anterior parts of the original lateral diverticula while the more posterior part of the ductus choledochus is formed from the hinder parts of the lateral diverticula without the intervention of a median pouch stage.

The lateral pouches merge into the pars hepatica mediana anteriorly and the distinction between these parts in this region is only possible through an examination of the tubule formation. Posteriorly, however, the proximal portion or stalk of each pouch is constricted and elongated to form the hinder part of a broad short duct connecting the pars ductus of the median pouch with the distal expanded portions of the lateral ones. This condition is illustrated by the transverse section shown in figure 16.

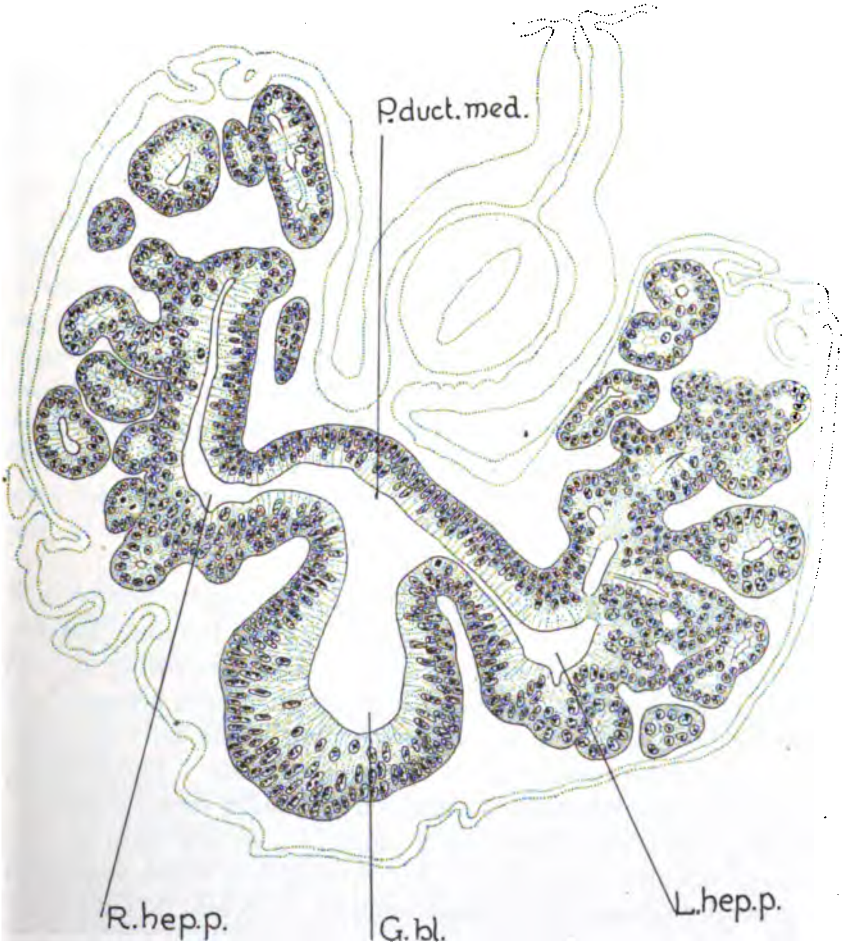

Fig. 16 Transverse section through the liver of an Acanthias embryo 15 mm. long (H.E.C. 227). × 125. *G.bl.*, gall bladder; *L.hep.p.*, left hepatic pouch. *P.duct.med.*, pars ductus mediana; *R.hep.p.*, right hepatic pouch.

The mesial surfaces of the lateral pouches are smooth except for some minor fissures and the gutter like spaces between them and the pars mediana are occupied by mesenchyma and the vitelline veins. The lateral surface of each pouch is almost obscured by the numerous hepatic tubules which arise from it. The dorsal growth of the pouches so evident in early stages has now come to an end and their dorsal margins hardly extend above the ventral surface of the stomach as is seen in figures 41 and 42. A number of large trunk-tubules arise from them. As in the pars hepatica mediana all the tubules of the lateral pouches tend to gather in certain fairly well defined groups. These consist, on either side, of an anterior and a posterior group, and the latter is less definitely subdivided into a dorsal and a ventral cluster. The tubules of the anterior groups spring from the dorsal half of the anterior part of the lateral pouch leaving a ventral area below which is smooth or occupied only by small tubules in the process of formation. The posterior group is much larger and its two subdivisions occupy the entire posterior half and hinder margin of the pouch. As will be seen from figure 41, these groups are not completely separated, as small and less developed tubules intervene in some places. The formation of these minor tubules continues until a much later period. The tubule groups of the anterior part of the left lateral pouch and of the left side of the pars hepatica mediana lie much closer together than those of the opposite side.

This early arrangement of the hepatic tubules into groups is of much importance for, as has been stated, while the main hepatic ducts are produced by the elongation and narrowing of the caliber of the lateral pouches, each group of tubules becomes isolated by the formation of a common stalk which later develops into one or more rami of the minor hepatic ducts. The arrangement of the tubule groups is expressed in tabular form in table 1.

A reconstruction, illustrated in figures 43, 45 and 46 of an embryo but 0.5 mm. longer than the preceding but which resembles in general anatomy the average embryo of 18 mm., shows more clearly the process of duct formation. The common bile duct is now three times as long as its greatest diameter and the pars ductus mediana has lost somewhat of its sac-like form and appears as an irregular dilated chamber which is broader in front than behind and receives the broad short cystic duct from below and the proximal parts of the lateral pouches from the sides. The pars hepatica mediana is somewhat elongated and is still broadly continuous with the pars ductus posteriorly. The formation of tubules from it has progressed considerably and now involves the ventral as well as the anterior surface. The division of this part of the median pouch into right and left segments by a vertical fissure is well marked. This fissure lies in the same sagittal plane as the left lateral wall of the pars ductus behind it, thus showing a marked shift to the left. On the right side the pars hepatica mediana is no longer confluent with the anterior part of the lateral pouch but is separated from it by narrow zone of tubule free surface (fig. 46). On the left side however the pars hepatica mediana extends far laterally and is continuous with the left lateral pouch posteriorly.

TABLE 1

Arrangement of hepatic tubule groups

Pars hepatica mediana	Right medial tubule group	
	Left medial tubule group	
Right hepatic pouch	Anterior right tubule group	
	Posterior right tubule group	Dorsal cluster
		Ventral cluster
Left hepatic pouch	Anterior left tubule group	
	Posterior left tubule group	Dorsal cluster
		Ventral cluster

The lateral pouches also show several changes. Their proximal stalks are elongated and the size of their distal expansions is much reduced. The connecting stalk of the left pouch with the pars ductus mediana is shifted so far forward that its posterior margin lies in the same transverse plane as the anterior margin of its fellow of the opposite side. The distal part of the left pouch is also farther separated from the posterior part of the median pouch than is the right.

The grouping of the tubules which arise from the lateral pouches is quite distinct except for the left medial and anterior left groups which have been rendered confluent by the vascular changes just discussed. The dorsal and ventral posterior clusters of the left side (fig. 45) are separate and the dorsal cluster which throughout early stages precedes the ventral one in development, is raised from the pouch surface and connected with it by a short broad pedicle. On the right side (fig. 46), the anterior right tubule group lies close to but is not fused with the right medial one. As on the opposite side, both dorsal and ventral clusters are distinct, and both are beginning to develop pedicles.

These differences between the right and left parts of the pars hepatica mediana and between the lateral pouches were present to some small degree in the preceding stage, but are more noticeable in this specimen and become more marked during later development. They are due primarily to the unequal size of the omphalo-mesenteric veins.

It is well known from the studies of Rabl ('92), Mayer ('89), Hammar ('93), and others, that in selachians there are at first two omphalo-mesenteric veins of almost equal size. Early in development however the right omphalo-mesenteric vein loses its connection with the area vasculosa and for a time ends blindly on the lateral wall of the yolk-stalk. Later a connection is formed between the posterior end of the right omphalo-mesenteric vein and the subintestinal vein by means of a channel lying to the right of the pancreas. During the period while the right omphalo-mesenteric vein ends blindly behind the blood from the yolk sac and from the subintestinal vein passes forward through the left omphalo-mesenteric vein alone, and this vessel in consequence becomes much enlarged. At this time the rotation of the gut from left to right about a longitudinal axis is in progress and the passageway for the vascular channel between the lateral hepatic pouch and the median hepatic pouch and fore gut is somewhat larger on the left side than on the right. This condition is shown both in the anterior view of the model of an embryo

10 mm. long and in figure 8 *A*, a cross section of the same specimen.

The effect of the enlargement of the left omphalo-mesenteric vein upon the liver anlage has already been described in part and will be considered farther in describing later stages. Passing along the left side of the median hepatic pouch or the common bile duct it pushes this structure to the right and shifts the left hepatic pouch or left hepatic duct anteriorly and laterally. This process is shown in an early stage by figure 17. At the same time the vessel upon encountering the left segment of the anterior and expanded part of the median hepatic pouch breaks up into several trunks which pass below and above the obstruction. One of the larger trunks passes through the dorsal part of the vertical cleft between the right and left anterior tubule groups or hepatic

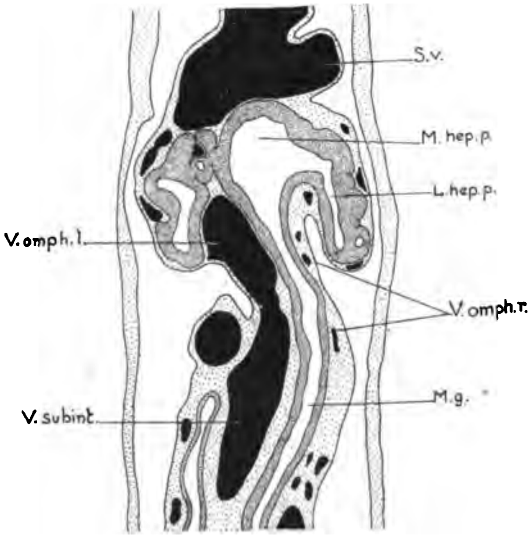

Fig. 17 Frontal section of the liver and mid gut region of an Acanthias embryo 13 mm. long (H.E.C. 226). × 40. *L.hep.p.*, lateral hepatic pouch; *M.g.*, mid gut; *M.hep.p.*, median hepatic pouch; *S.v.*, sinus venosus; *V.omph.l.*, left omphalo-mesenteric vein; *V.omph.r.*, right omphalo-mesenteric vein; *V.subint.*, subintestinal vein.

rami and gradually enlarging this space presses the tubules apart until it forms one of the larger channels of the vein.

The right omphalo-mesenteric vein, after establishing its posterior connection with the subintestinal vein, which it does when the embryo reaches a length of about 10 mm., grows rapidly, although it never equals in size that of the opposite side, and does not affect the position of the biliary apparatus to any great degree. At first the right and left omphalo-mesenteric veins pass forward to meet in front of the anterior portion of the median hepatic pouch but in later stages the vessels become confluent behind and below the anterior part of the median hepatic pouch or its derivative, thus forming a large sinus which increases the effect already begun by the left vitelline vein, viz., shifting the common

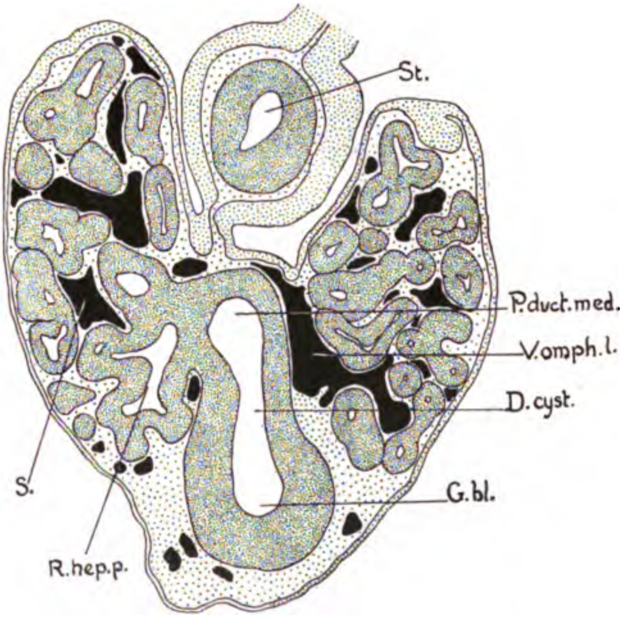

Fig. 18 Transverse section of an Acanthias embryo, 15.5 mm. long (S.C. 1). × 100. *D.cyst.*, cystic duct. *G.bl.*, gall bladder; *P.duct.med.*, pars ductus mediana; *R.hep.p.*, right hepatic pouch; *S.*, sinusoids of right omphalo-mesenteric vein; *St.*, stomach; *V.omph.l.*, left omphalo-mesenteric vein.

bile duct and cystic duct to the right and the left hepatic forward and laterally. This condition is shown by a frontal section of a much older embryo in figure 19.

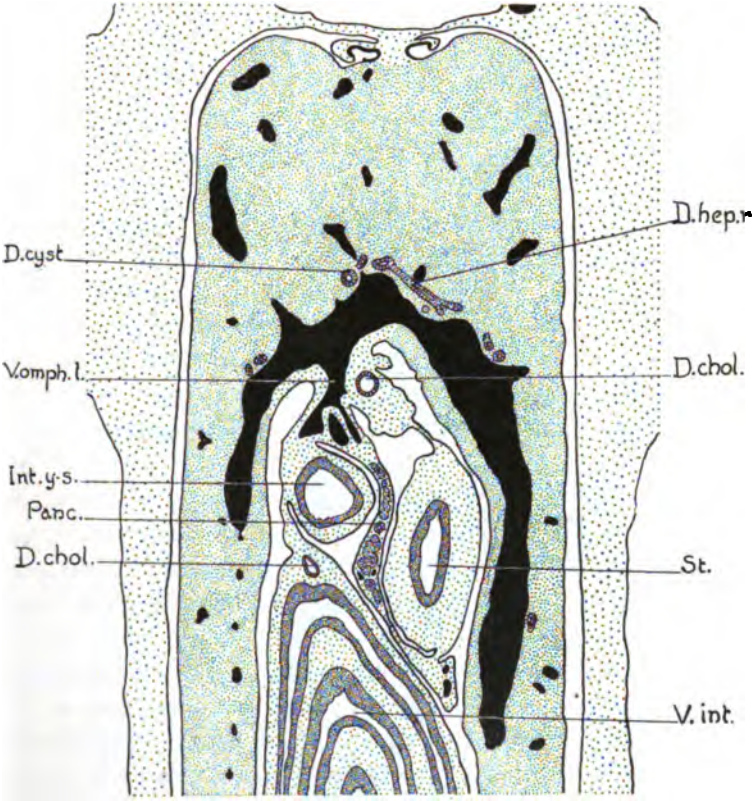

Fig. 19 Frontal section of an Acanthias embryo 41 mm. long (H.E.C. 371). × 40. *D.chol.*, ductus choledochus; *D.cyst.*, cystic duct; *D.hep.r.*, right hepatic duct; *Int.y.s.*, internal yolk sac; *Panc.*, pancreas; *St.*, stomach; *V.int.*, valvular intestine; *V.omph.l.*, left omphalo-mesenteric vein.

An embryo 20.5 mm. in length shows sufficient differentiation of the embryonic hepatic structures to permit the introduction of the adult terminology in describing them. As will be seen from a cross section through the anterior end of the gall bladder of a specimen of nearly the same stage (fig. 20), both lateral and median pouches are reduced in diameter and the tubule groups, in part at any rate, are connected with the latter by short ducts. A reconstruction of the biliary apparatus of this stage is shown in figures 47, 48 and 49.

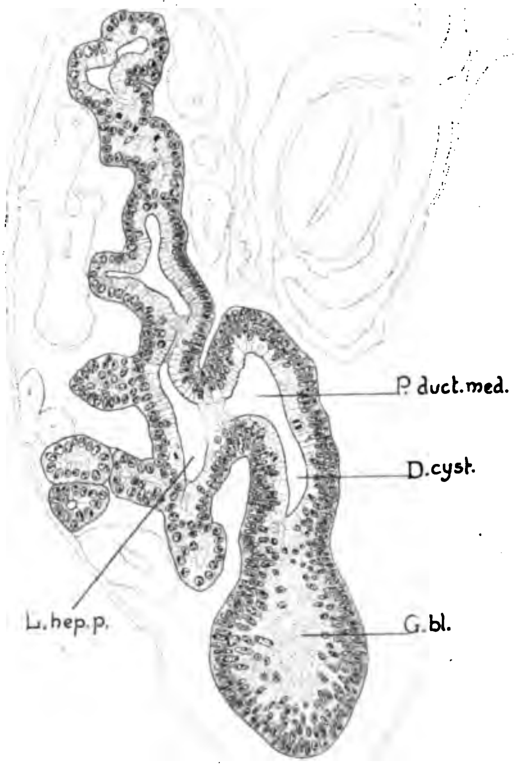

Fig. 20 Transverse section of an Acanthias embryo 19 mm. long (S.C. 2). × 100. *D.cyst.*, cystic duct; *G.bl.*, gall bladder; *L.hep.p.*, lateral hepatic pouch; *P.duct.med.*, pars ductus mediana.

The pars ductus mediana, or as the structure may now be termed, the terminal part of the ductus choledochus, is tubular in form. The pars hepatica mediana is reduced in size antero-posteriorly and expanded laterally. The vertical cleft dividing it into right and left parts lies lateral to the left wall of pars ductus and is accentuated by the two short ducts which arise on either side of it. These ducts are the right and left medial hepatic rami and are the derivatives of the right medial and left medial tubule groups respectively. As yet they are very short and large calibered and break up almost immediately into a number of hepatic tubules. Two tubules arising immediately ventral to the left medial ramus probably represent the remains of the anterior left tubule group whose fusion with the left medial group has been described. Three additional tubules of large caliber arise from the anterior and ventral surface of the pars hepatica mediana. They may be derived from the right or left medial group but probably have arisen direct from the pouch wall after the main tubule groups were established.

The left hepatic pouch or duct as we may now term the structure, takes origin entirely from the lateral part of the posterior surface of the pars hepatica mediana, having been entirely separated from the pars ductus mesially. Thus it is already evident that the adult hepatic duct upon this side is made up of two elements, a proximal and transverse part derived from the left part of the pars hepatica mediana and a distal and longitudinal part formed from the left lateral pouch proper. On the opposite side the pouch or duct arises, as in preceding stages, from the lateral surface of the pars ductus or common bile duct. A distinct groove separates the anterior boundary of the right duct from the pars hepatica mediana. Also as in earlier stages the left duct is widely separated from the pars ductus while the right duct lies quite close to its opposite side. The condition of the anterior left tubule group has already been described and on the right side the anterior group is connected with the main hepatic duct by a distinct neck, the anterior right hepatic ramus, which is directed dorsally. On the left side the dorsal posterior cluster or ramus is no farther developed than before, but the ventral cluster possesses a short duct which extends posteriorly and bifurcates into upper and lower branches. On the right side the dorsal posterior cluster is represented by two ducts, the upper one being particularly prominent and directed posteriorly. The posterior ventral cluster arises from a very short broad diverticulum of the pouch and breaks up into five large tubules, the largest of which is directed posteriorly.

In an embryo of 20.6 mm. (H.E.C. 1494, No. 28, Normal plate series) the hepatic ducts are so completely formed that their origin from pouches and tubule groups would hardly be surmised from the reconstruction of them seen in figure 50. The pars ductus mediana is not separable from the more posterior part of the ductus choledochus and the left part of the pars hepatica mediana and the left lateral pouch form together one duct, the left hepatic, which arises from the lateral surface of the ductus choledochus and curves first laterally and then backward. The distinct angle between the transverse and longitudinal parts of the duct is the point of union of the two elements which form it. The right medial hepatic ramus arises at the union of the left hepatic duct with the ductus choledochus. Immediately to the left of this is a smooth narrow segment of the left hepatic duct which lies between two branches of the left vitelline vein. To the left of this segment lies a dorsal duct, the left medial ramus, and below and lateral to it is the anterior left ramus. These represent the anterior left and left medial tubule clusters respectively. The small tubules which are seen in the anterior view of the model arising between these two ducts are probably derived in part from both tubule groups which, it will be remembered, were somewhat fused in earlier stages.

The right hepatic duct is much shorter than the left. It takes origin from the anterior end of the ductus choledochus and curves backward at once. A very large dorsal ramus arises from its upper surface just distal to its connection with the ductus choledochus. This is the anterior right hepatic ramus and corresponds to the tubule group of the same name in younger embryos. Aside from this and one small tubule arising from the anterior surface, the proximal part of the right hepatic duct is smooth. Near its distal end it gives off several minor tubules. The dorsal ones represent the dorsal posterior tubule cluster while the ventral ones are derived from the ventral posterior tubule cluster as is also the most distal part of the duct itself. By this stage all the minor hepatic rami are established and no new tubules arise from the ducts direct, all further increase in hepatic parenchyma being due to growth in the hepatic trabeculae proper.

The later histories of the major and minor hepatic ducts can be most easily undertaken separately. Up to the stage just described the gradual shifting of the anterior end of the ductus choledochus to the right has been a constant feature. This process becomes more noticeable with the distinct differentiation of the hepatic ducts and by the time the embryo reaches a length of from 25 to 28 mm., the anterior end of the duct may be so rotated that the original anterior surface faces the right and the left surface appears as the anterior one. The rotation though always present is not always so extreme as this type which is shown in figures 51 and 52. This process is brought about by a distinct enlargement of the sinus formed by the omphalo-mesenteric veins which lies below and to the right of the ductus choledochus. The rapid growth of this sinus is in turn due to changes in the hepatic trabeculae. Until the embryo reaches the length of from 25 to 28 mm. the trabeculae form a wide meshed network and the blood is able to flow around them through sinusoids of large caliber. From this stage on however the trabeculae increase rapidly in size and the surrounding sinusoids are reduced to extremely small vascular channels.[5] The blood is thus directed in a large part into the omphalo-mesenteric veins and their caliber is considerably increased.

The main hepatic ducts are markedly influenced by this rotation. The left one is carried forward until almost its entire length lies in the transverse plane of the embryo and only the distal end with the tubules arising from it project backward while the right hepatic duct extends directly backward with little or no lateral course (figs. 21, 51, 52 and 54). Soon after the rotation of the anterior end of the ductus choledochus takes place the cystic duct, which until this time has joined with the ductus choledochus from below, is carried forward and upward along with the gall bladder until it joins with the anterior surface of the ductus choledochus and appears at first sight as an anterior extension of that structure. This process, illustrated in figures 22, 23, 24, 25 and 54, is brought about in part at least by the growth of the internal yolk sac and is discussed in more detail in the section upon the development of the gall bladder. A distinct forward arch appears in the proximal portion of the main hepatic ducts so that instead of entering the ductus choledochus at right angles to its longitudinal axis as in younger stage, they extend backward on either side of it for a short distance and then join with it very obliquely. This arch may be partially brought about by the hepatic ducts being forced forward by the same agency as that influencing the cystic apparatus at this time but probably it is also due in part to the actual shifting backward of the ostia of the hepatic ducts along the ductus choledochus.

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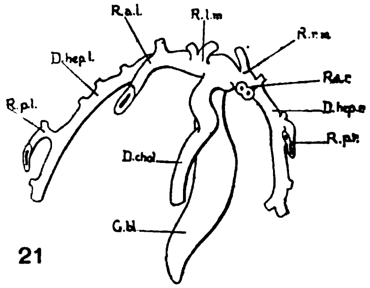
[5] This fact has already been recognized and recorded by Minot ('00).

Up to the stage represented by the embryo of 20.6 mm., the hepatic ducts were described with little difficulty for they pursue much the same course in all the specimens which were examined. However, the reduction in size of the distal end of the ductus choledochus and the shiftings which it and the hepatic ducts undergo modify considerably the position of some of the minor ducts and to varying degrees in different specimens. This applies particularly to the anterior left and right lateral rami and the left and right medial rami. The posterior dorsal rami remain fairly constant in position at the juncture of the transverse and posterior parts of their respective trunks, and the posterior ventral rami, while giving rise to new short minor sprouts in their course, seem fairly regular.

Of the several types observed in embryos and specimens of the pup stage, the commonest and simplest one is that in which rotation of the ductus choledochus is present but not extreme and in which the embryonic arrangement of the rami is to a large extent retained. This form is illustrated by the graphic reconstruction of two embryos, one 28 mm. and one 41 mm. in length illustrated in figures 21 and 22.

The variants from this type so far as observed have been such as might be expected from farther rotation of the anterior part of the ductus choledochus. If this process is extreme all those rami which arise from the anterior surface of the pars hepatica



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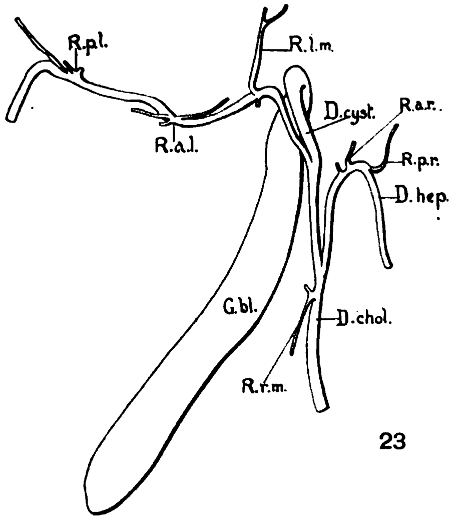

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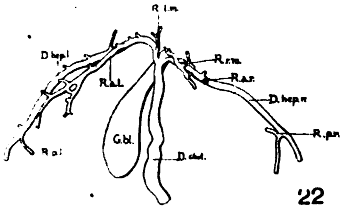

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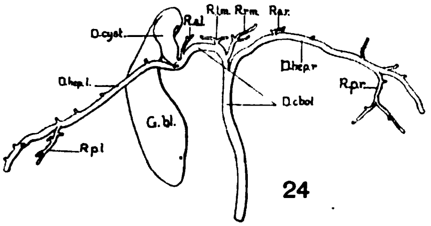

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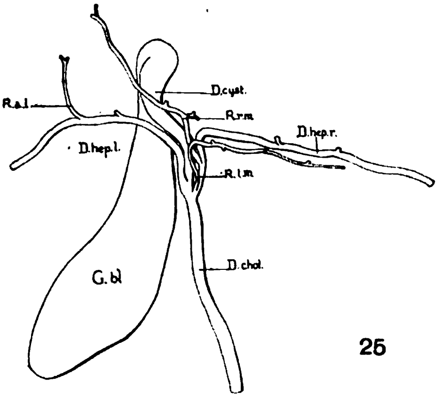

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Fig. 21 Graphic reconstruction (dorsal view) of the gall bladder and liver ducts of an embryo 28 mm. long (H.E.C. 221). × 20. For abbreviations, see figure 22.

Fig. 22 Graphic reconstruction (dorsal view) of the gall bladder and liver ducts of an embryo 41 mm. long (H.E.C. 371). × 20. *D.chol.*, ductus choledochus; *D.cyst.*, cystic duct; *D.hep.l.*, left hepatic duct; *D.hep.r.*, right hepatic duct; *G.bl.*, gall bladder; *R.a.l.*, anterior left hepatic ramus; *R.a.r.*, anterior right hepatic ramus; *R.l.m.*, left medial hepatic ramus; *R.r.m.*, right medial hepatic ramus; *R.p.l.*, posterior left hepatic ramus; *R.p.r.*, posterior right hepatic ramus.

Fig. 23 Graphic reconstruction (dorsal view) of the gall bladder and liver ducts of an embryo 18 cm. in length. × 10. For abbreviations, see figure 22.

Fig. 24 Graphic reconstruction (dorsal view) of the gall bladder and liver ducts of an embryo 60 mm. in length (H.E.C. 409). × 20. For abbreviations, see figure 22.

Fig. 25 Graphic reconstruction (dorsal view) of the gall bladder and liver ducts of an embryo 86 mm. in length (H.E.C. 410). × 20. Only the proximal part of the hepatic ducts are represented. For abbreviations, see figure 22.

mediana and apparently even the anterior left ramus may be carried dextrally until they face the right. Apparently also any intermediate step between this extreme and the embryonic type described above may exist. When rotation first takes place and before the cystic duct begins to shift upward and forward the ostia of the minor anterior ducts remain quite close to that of the main left hepatic duct. With this change however the curve formed by the extreme anterior part of the ductus choledochus and the cystic duct is gradually obliterated so that the two ducts come to form together a slender tube which extends anteroposteriorly in frontal plane, and the ostia of the rami which formerly were on the anterior surface of this curve come to lie on one side or the other of duct and are separated by its dorsal surface. Figures 51 and 52 are of a reconstruction of the biliary apparatus at a stage immediately after a distinct dextral rotation of the ductus choledochus has taken place and before the dorso-anterior migration of the cystic duct and gall bladder is very noticeable. Had this specimen continued its growth, if one may judge from reconstructions of later embryos, the cystic duct would probably be carried forward and upwards in such a way that it would intervene between the left medial ramus and the left main hepatic duct in such a way that it would receive the opening of the former on the right hand side and the latter on the left. Figures 53 and 54 of an embryo 33.1 mm. in length shows a somewhat later stage in which the cystic duct is being forced forward and the right medial ramus which formerly lay almost in the median line is being carried over to the right side of the duct.

Some reconstructions which illustrate the results brought about by the above processes may be illustrated here as they also show the changes which take place in the major ducts and gall bladder in the later periods of embryonic development.

Figure 23 of a very late embryo 18 cm. in length shows a variant in which the left medial ramus has remained attached to the main left hepatic duct but in which the right medial ramus arises from the ductus choledochus. Except for this change, the rami have remained in their early embryonic position. Figure

24 of an embryo 60 mm. in length shows a specimen in which the rotation of the ductus choledochus to the right must have been very great indeed for it still shows an almost right angled curve in that direction at this late stage and the right medial, the left medial, and the anterior left rami all arise from it between the ostia of the cystic and left hepatic and the right hepatic duct. The figure of an embryo of 86 mm. (fig. 25) shows the most extreme case of the series in which both the left and the right anterior rami arise from the right hepatic duct, the latter almost at its ostium. I can only explain this case on the supposition that the posteriorly directed proximal segment of each the right and left main hepatic ducts is derived in part by a splitting off from the ductus choledochus and that the left medial ramus originally connected with an area of the right side of the ductus choledochus which was later incorporated in the distal end of the right lateral hepatic duct.

That other variations in the arrangement of these hepatic rami may occur both in embryo and adult is very probable, but it is to be expected that they will all be of the same general type as those described above, viz., such as may be brought about by the progression of their ostia from left to right. Besides the variation in position of ostia, there are also noticeable ones in the size of the various elements. This seems to be compensatory in that the reduction in extent of any element is accompanied by an increase in size and complexity of its neighbors. Occasionally one element may be replaced by two or more smaller ones and minor rami are commonly found irregularly placed in regard to the larger ones. These minor sprouts as already stated arise either from tubules intermediate in position to the main tubule groups or from such tubules as arise after these groups are somewhat separated from the main ducts by pedicles. A scheme of the origin of the minor ducts or rami will be found in the general summary in table 3.

III. DEVELOPMENT OF THE GALL BLADDER AND CYSTIC DUCT

The gall bladder has been described in the first section of this paper as arising as a median outpouching of the anterior wall of the yolk-stalk immediately below the point where that structure becomes continuous with the floor of the fore gut. The ventral zone of the lateral walls and the floor of this part of the gut at this early stage are a part of the hepatic area and that part of the anterior wall of the yolk-stalk from which the gall bladder arises is later incorporated in the gut behind the liver as the yolk-stalk becomes constricted antero-posteriorly. The time of appearance of the anlage of the gall bladder is somewhat later than that of the liver as has been noted by Laguesse ('93) and Debeyra ('09). It seems possible, from these observations, to regard the gall bladder in this form as derived from a secondary pouch which is formed in the floor of the archenteron immediately behind the liver pouch proper. If this interpretation be correct, an anterior shifting of the gall bladder by which its opening reaches its later position at the anterior end of the ductus choledochus in front of the openings of the hepatic ducts is to be expected. Evidences of such a shifting are not wanting. The early demarkation of the gall bladder has already been described in the preceding section, and has been seen to consist of the formation of dorsal longitudinal grooves and a posterior and ventral vertical one by which the gall bladder is cut off from the anterior wall of the yolk-stalk behind and remains attached to the liver pouch above by a transversely constricted neck which extends along its entire dorsal surface. The anterior end of this neck is less constricted and represents the cystic duct. These changes are illustrated in figures 33, 35 and 37, and are also shown in *B* of figure 26 below.

These changes are even more marked in an embryo of 10 mm. illustrated in figures 39 and 40, and in *C* of figure 26. Along with them is a considerable lateral expansion of the gall bladder and a curious growth of its anterior wall which produces a pointed process which projects forward below the ventral wall of the median hepatic pouch. The gall bladder in this and the preceding stages which follow its separation from the anterior wall of the yolk-stalk behind lies a little to the left of the median line. This asymmetric position has already been discussed in Part I. It is associated with the rotation of the gut in connection with the formation of the spiral valve.

When the liver reaches the stage represented in figures 41 and 42, of an embryo of 15 mm., the gall bladder is so far separated from it as to form a thick walled ovoid sac, the posterior half of which is rounded and free and the anterior end drawn out into a cone shaped projection. It is still broadly attached to the median liver pouch above. The posterior end extends downward so that the long axis of the sac is no longer parallel with that of the median liver pouch above it.

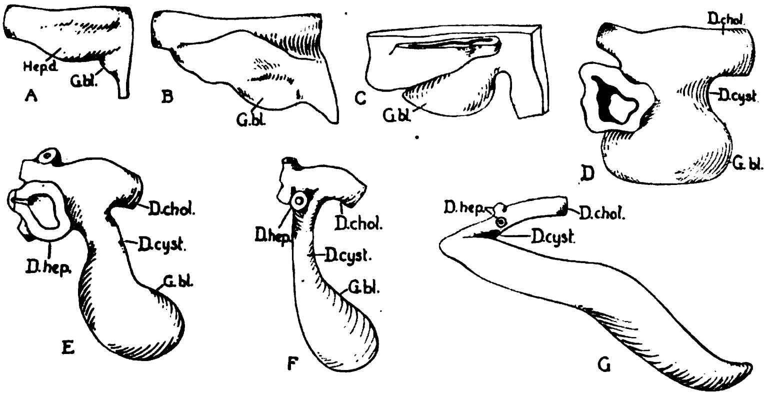


Fig. 26 A series of semi-schematic figures of the development of the gall bladder and cystic duct. *A*, embryo of 7 mm.; *B*, embryo of 7.5 mm.; *C*, embryo of 10 mm.; *D*, Embryo of 15.5 mm.; *E*, embryo of 19 mm.; *F*, embryo of 20.6 mm.; *G*, embryo of 33.1 mm. All from plastic reconstructions with the exception of *A*, which is based upon longi-sections. *D.chol.*, ductus choledochus; *D.cyst.*, cystic duct; *D.hep.*, hepatic duct; *G.bl.*, gall bladder; *Hep.d.*, hepatic diverticulum.

The gall bladder in an embryo of 15.5 mm. (figs. 45 and 46 and *D*, fig. 26) is smaller relatively, and the connection between it and the liver above is drawn out into a short broad stalk, the cystic duct. The pointed anterior extremity is less noticeable than in the two preceding specimens and from this time on no evidences are seen of it. In this specimen, also, the gall bladder lies to the left of the median line and the cystic duct is inclined to the right as well as upward to meet the median hepatic pouch or ductus choledochus.

At 19 mm., the gall bladder is widely separated from the ductus choledochus and inclined at a distinct angle to it. The cystic duct is decidedly elongated and extends upward to the right and decidedly forward to meet the ductus choledochus. This elongation is due in part to the increase in size of the left omphalomesenteric vein, which lies in this region between the gall bladder below and the ductus choledochus above, and perhaps also in part to the great growth of the hepatic trabeculae which takes place at this time. Probably the increased length of the duct is derived in part at least from the lower part of the ductus choledochus from which the anterior end of the cystic duct seems to be separating. This separation is peculiar in that it takes place mesially more rapidly than laterally, thus forming a distinct pocket which is bounded below by the cystic duct, above by the floor of the ductus choledochus and laterally by the sides of these structures which are still confluent. This formation is only temporary.

At 20.6 mm. (fig. 50, and fig. 26, *F*) the cystic duct is still more elongated and joins the floor of the ductus choledochus nearly at right angles to it. The gall bladder is shifted downward until its long axis is almost in the vertical plane of the embryo and at right angles to the ductus choledochus. It is drawn out until the sac appears as little more than the expanded end of the cystic duct.

The shifting of the ductus choledochus and the rotation of its anterior end, described on page 371, now takes place, and the cystic duct and a part of the gall bladder share in this displacement. The posterior half of the gall bladder remains almost in the position occupied in the preceding stage or is shifted a little dorsally, while the anterior half and the cystic duct form together an abruptly curved tube which extends to the right and dorsally. In this way there is formed a distinct flexure in the middle part of the gall bladder. This is illustrated by an embryo 28 mm. in length (figs. 51 and 52).

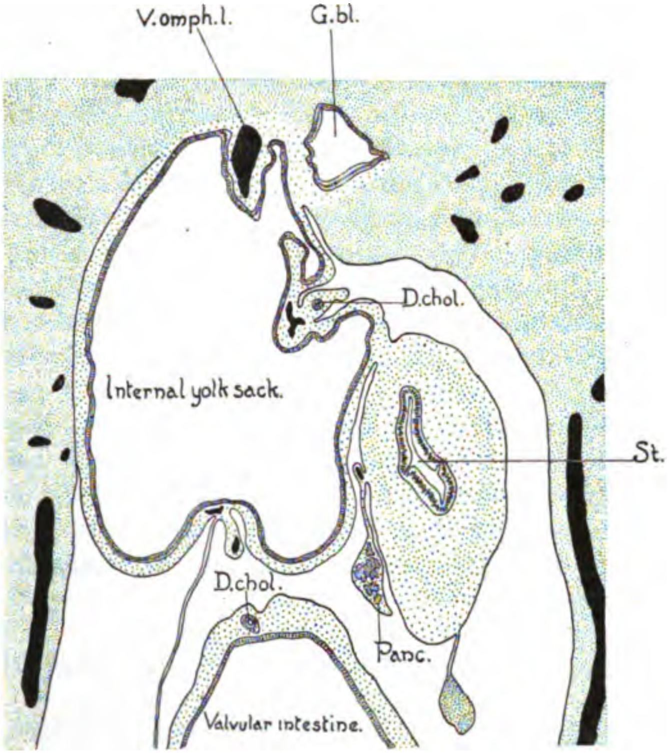


Fig. 27 Frontal section of an Acanthias embryo 86 mm. long (H.E.C. 410). × 40. *D.chol.*, ductus choledochus; *G.bl.*, gall bladder; *Panc.*, pancreas; *St.*, stomach; *V.omph.l.*, left omphalo-mesenteric vein.

Along with the changes described above comes a very extensive alteration in both the form and position of the cystic apparatus which is apparently produced by the development of the internal yolk sac. This structure begins to develop from the vitelline duct just internal to the ventral body wall in embryos from 25 to 30 mm. in length. An early stage is seen in section in figure 17. It grows rapidly and its anterior end pushes forward between and below the stomach and left and cystic lobes of the liver on one side and the right lobe of the liver on the other. The frontal section shown in figure 27 illustrates its size and relations in an embryo 60 mm. in length.

In this way the yolk sac presses upon the right side and lower surface of the gall bladder. The first effect of this pressure is to force the posterior part of the gall bladder upward so that this structure retraces in part the path of downward extension which it followed in earlier stages (figs. 22, 23, 24, 25 and 26 *G*). At the same time the gall bladder is elongated and somewhat flattened vertically and its anterior end is pushed upward and forward. At first this only causes an abrupt curve in the cystic duct (fig. 54), but as this process is continued the gall bladder is finally forced anteriorly and dorsally beyond the distal end of the ductus choledochus and the cystic duct proceeds backwards over its dorsal surface to join that structure. In this way the ostium of the cystic duct which was originally in the floor of the ductus choledochus is rotated to its anterior surface and the two ducts together form one continuous tube sometimes called the ductus cystocholedochus, the juncture of the two elements of which is at the ostia of the hepatic ducts. This is also illustrated in figures 22, 23, 24, 25 and 26 *G*.

IV. DEVELOPMENT OF THE DUCTUS CHOLEDOCHUS

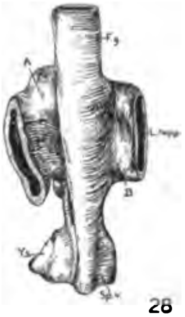
The development of the ductus choledochus is better known than that of any other part of the biliary apparatus in elasmobranchs. For our information in regard to the earlier stages we mainly have to thank Hammar ('93), Brachet ('96), Mayr ('97) and Choronschitzky ('00) while the later stages have been most successfully studied in connection with the development of the spiral valve by means of the reconstruction method by Rückert ('96, '97).

The development of this structure in Acanthias has been to some extent described incidentally in connection with the account of the early stages of the liver and of the hepatic and cystic ducts and gall bladder in this paper so that only a short summary need be given here.

In Acanthias, the ductus choledochus when fully formed is a complex consisting of three embryonic elements: (1) a proximal or posterior segment derived from the floor of the duodenum and valvular intestine; (2) a middle segment formed from the 'pars ductus lateralis' of the primitive lateral hepatic diverticula; (3) a distal and anterior segment which is differentiated from the posterior portion or 'pars ductus mediana' of the secondary median hepatic pouch. Thus the middle and distal portions of the duct are both derived from the primitive lateral diverticula, at a very early stage and are truly hepatic in origin while the proximal or posterior part is formed from the archenteron at a much later stage after the duodenum and valvular intestine. In the fully formed fish the latter segment forms by far the greater part of the duct being represented by practically all the extra hepatic portion.

The differentiation of the posterior part of the lateral hepatic diverticula into the pars ductus lateralis has already been described in Part I, as well as the early demarkation of the pars ductus mediana from the median hepatic pouch. At 10 mm. the pars ductus lateralis forms the slightly expanded ventral half of the rather constricted segment of archenteron which connects the median hepatic pouch and the gut above it with the yolk-stalk and mid gut posteriorly (figs. 39 and 40). The pars ductus lateralis is thus continuous anteriorly and below with the gall bladder and median pouch and above with the dorsal part of this stalk which later forms the duodenum. On the dorsal surface of the median pouch (fig. 28) is an oblique ridge which extends from its left anterior to right posterior angle and maps off the area of this structure which later becomes the distal part of the ductus choledochus. At 15 mm. (figs. 41 and 42) both the median hepatic pouch and the pars ductus lateralis behind it are completely cut off from the gut above. The latter now forms a short wide duct about 0.1 mm. in length which extends backward a little obliquely from left to right and joins with the floor of the gut a little to the right of the median line. In cross section the duct is equal in diameter to that segment of gut, the duodenum, with which it joins and is triangular in outline with the apex of the triangle directed upward. It thus shows a trace of the pouch-like structure from which it arises but at a little later stage it becomes circular or oval in cross section. The pars ductus mediana is still sac-like and like the duct behind it is inclined a little from left to right.

In 15.5 to 18 mm. embryos the part of the duct derived from the pars lateralis is nearly twice as long as in the above specimen (figs. 45 and 46) and extends directly backward joining the posterior end of the duodenum in the median line. Its diameter is about 0.1 mm., being distinctly less than that of the earlier stage and about one-half that of the gut which it joins. The pars ductus mediana is rapidly taking on a duct-like form although this feature is somewhat obscured by the large ducts which arise from it.



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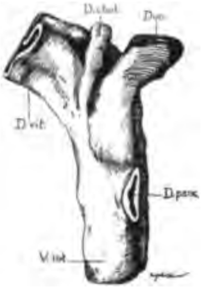

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Fig. 28 Dorsal view of a reconstruction of an Acanthias embryo 10 mm. long (S.C. 20). × 36. Lateral and anterior views of this model are shown in figures 38 and 39. *A*, anterior part; *B*, posterior part of ridge marking the formation of the anterior part of ductus choledochus from the pars ductus medialis of the median liver pouch. *F.g.*, fore gut; *L.hep.p.*, lateral hepatic pouches which are cut away dorsally; *Sp.v.*, spiral valve; *Y.s.*, yolk stalk.

Fig. 29 Reconstruction of the mid gut region of an Acanthias embryo 20.5 mm. long (S.C. 5). × 50. *Duo.*, duodenum; *D.chol.*, ductus choledochus; *D.panc.*, pancreatic duct; *D.vit.*, vitelline duct; *V.int.*, valvular intestine.

In the stages which now follow, as is shown in figures 51, 52 and 54, the duct rapidly increases in length due to the elongation of its extra-hepatic portion. This growth seems to be derived from the floor of the digestive tube for there extends in the floor of the gut posterior to the ostium of the duct a deep groove which is lined with epithelium of the same nature as that of the duct itself. At 15.5 mm., the duct joins the gut near the posterior end of the duodenum. At 20.5 mm., as is shown in figure 29, this opening lies at the point where the duodenum becomes continuous with the valvular intestine in the angle between the latter structure and the vitelline duct. The duct lies below and to the right of the median line of the duodenum, but as this structure is continuous only with the left side of the valvular intestine (the right side of which is continuous with the vitelline duct) the ductus choledochus joins the valvular intestine directly in the median line of the gut.

From this time to until the embryo reaches a length of approximately 25 mm. the duct grows steadily backward and its ostium remains in the median line. It grows past the opening of the vitelline duct passing it to the left but never overtakes the ostium of the pancreatic duct.

When the embryo reaches the length of about 25 mm., however, the duct becomes involved in the twisting process of the spiral valve and is carried to the left. At 28 mm. its opening is in the middle of the right side of the valvular intestine and at 37 to 48 mm. it lies at the junction of the superior and right surfaces. In embryos 60 to 80 mm. long, the duct enters the intestine on its dorsal surface at the edge of the first turn of the spiral valve, and in the new-born fish the opening lies at the junction of the left side with the dorsal surface of the intestine. These changes in position as well as the comparative diameter of the duct at different stages are shown in table 2.

Along with the elongation of the ductus choledochus come several modifications of its position besides the posterior shifting just described. The intrahepatic portion is affected by the growth of the internal yolk sac and by the vascular changes already discussed in connection with their effect upon the position of the hepatic and cystic ducts. This part of ductus choledochus is first arched upward in a stiff almost semi-circular curve by the formation of a venous sinus below it and its extreme anterior end is rotated to the right. These changes are shown in figures 51, 52 and 54. In later stages and in the new-born fish these changes are less noticeable, being probably compensated by the growth of the hepatic parenchyma, but in the new-born fish as well as the adult the distal end of the duct lies distinctly to right of the median line. As the duodenal flexure is developed, the middle part of the duct is also directed sharply ventrally along with the change in position of the segment of the gut which it joins. This ventral curve appears in embryos of 18 to 20 mm. and is at first a gradual one but becomes sharper and more pronounced in older embryos. In the course of this curve the duct is also pushed to the right by the vitelline vein which lies beside it. Minor short flexures appear in later stages near the anterior and posterior ends of the duct and in its duodenal curve as well. They are not constant in position, shape or number. The duct

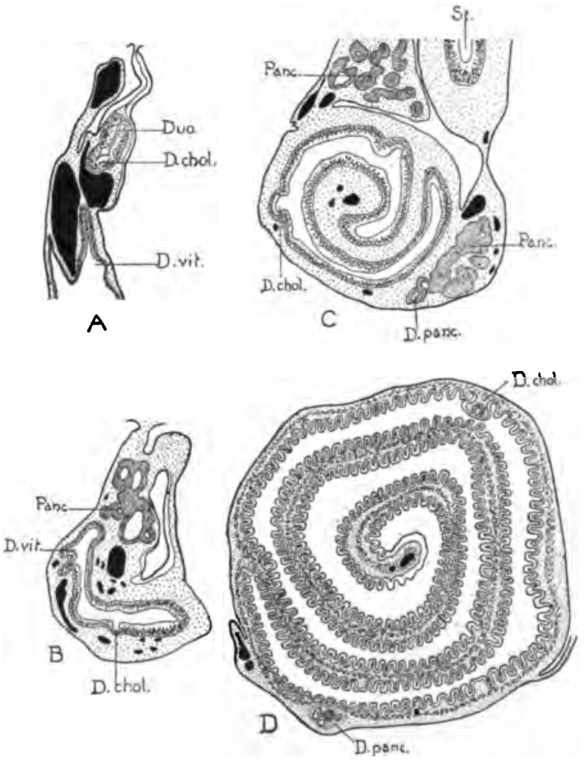

Fig. 30 A series of transverse sections of Acanthias embryos of different ages at the level of the ostium of the ductus choledochus. *A*, embryo 15.5 mm. long (S.C. 1); *B*, embryo 20.6 mm. long (H.E.C. 1494); *C*, embryo 32.2 mm. long (H.E.C. 1662); *D*, embryo 180 mm. long (S.C. 51) all except *D* × 28; *D* × 8. *D.chol.*, ductus choledochus; *D.panc.*, pancreatic duct; *D.vit.*, vitelline duct; *Duo.*, duodenum; *Panc*, pancreas; *St.*, stomach.

is much reduced in diameter between the time when it is first formed and when it begins it growth backward along the base of the valvular intestine. After this phase begins there is but little change in caliber. In the intrahepatic and preduodenal regions the duct is circular in trans-section, posteriorly it is flattened transversely until elongately oval in trans-section.

Table 2 shows numerically the growth of the duct and the changing relations of its ostium. Some error is doubtless introduced in the calculation of the length of the duct from cross sections as is done here, but Acanthias embryos of 18 mm. and over shrink comparatively little in imbedding and that shrinkage seems to be symmetrical so that the value of the figures is not seriously impaired. The 'extra-hepatic length' is taken from the point where the duct turns sharply downward immediately after leaving the liver to the point where its lumen becomes continuous with that of the gut. It is not possible to determine this point exactly in specimens under 20 mm. in length so only the total length of the duct has been given in such cases.

TABLE 2

Growth and relations of the ductus choledochus

DESIGNATION	LENGTH OF EMBRYOS	LENGTH OF DUCT			DIAMETER OF DUCT	RELATION OF OSTIUM TO OSTIUM OF VITELLINE DUCT	RELATION OF OSTIUM TO OSTIUM OF PANCREATIC DUCT	POSITION OF OSTIUM IN INTESTINAL WALL
		Intra hepatic	Extra hepatic	Total				
	mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	
H.E.C. 227	15.0			0.11	0.15	0.23 ant.	0.40 ant.	Entire ventral surface
S.C. 1	15.5			0.22	0.11	0.20 ant.	0.32 ant.	Entire ventral surface
S.C. 2	19.0			0.16	0.9	0.14 ant.	0.33 ant.	Entire ventral surface
H.E.C. 1494	20.6	0.22	0.18	0.40	0.8	Same plane	0.12 ant.	Mid-line of ventral surface
H.E.C. 1492	24.7	0.10	0.32	0.42	0.8	0.10 post.	0.35 ant.	Junction right and middle thirds of ventral surface
H.E.C. 1357	28.0	0.70	0.62	0.72	0.5	0.40 post.	0.25 ant.	Junction lower and middle thirds of right surface
H.E.C. 1652	32.2	0.16	0.86	1.02	0.5	0.60 post.	0.29 ant.	Junction lower and middle thirds of right surface
H.E.C. 363	37.0	0.36	1.08	1.44	0.7	0.80 post.	0.46 ant.	Junction right and dorsal surfaces.
S.C. 11	47.3	0.72	1.10	1.82	0.7	0.91 post.	1.76 ant.	Junction right and dorsal surfaces
H.E.C. 1882	95.0	1.20	5.0	6.2	0.7	2.4 post.	1.2 ant.	Mid-line of dorsal surface

V. GENERAL SUMMARY

The observations here recorded may be summarized as follows:

1. The liver arises in Acanthias embryos of from 20 to 25 segments as a pair of shallow lateral diverticula from the lateral walls of the ventral half of the gut. These diverticula extend both behind and in front of the anterior wall of the yolk-stalk.

2. The growth of the fore gut posteriorly through the coalescence of the lateral walls of the yolk-stalk causes the lateral hepatic diverticula to lie mainly in front of the anterior wall of the yolk-stalk in later stages.

3. The median ventral liver pouch described by Balfour and others is, in Acanthias at least, a secondary structure produced by the fusion of the anterior ends of the primary lateral diverticula.

4. Three distinct secondary parts are derived from each primitive lateral hepatic diverticulum. The anterior portion goes to the median hepatic pouch. The upper part of the middle portion forms the lateral hepatic pouch. The posterior part goes to form a posterior connecting segment between the liver and gut. This has been termed the pars ductus lateralis and later becomes a part of the ductus choledochus.

5. At an early stage the liver shares in the left to right rotation which produces the spiral valve in the intestine and the lateral vitelline groove in the yolk stalk.

6. The median hepatic pouch is somewhat differentiated into two parts: an anterior one, the pars hepatica mediana to which the lateral pouches are mainly attached and which gives rise to hepatic trabeculae, and a posterior one, the pars ductus mediana, which forms the anterior part of the ductus choledochus.

7. The anterior part of the left hepatic duct is formed from the left hepatic pouch and the left part of the pars hepatica mediana. The anterior part of the right hepatic duct is formed from the right hepatic pouch alone.

8. The hepatic ducts and ductus choledochus are very markedly rotated to the right about a vertical axis in a comparatively late stage. This rotation is probably due to the great growth of the left omphalo-mesenteric vein and the formation of a venous

sinus below and to the left of the anterior end of the ductus choledochus.

9. The difference in size of the right and left omphalo-mesenteric veins may be due to the longitudinal rotation of the gut mentioned in section 5 of this summary. By this rotation the space between the left hepatic pouch and the median pouch and gut is somewhat increased while the corresponding space on the opposite side is decreased.

10. A third shifting of the duct system and the gall bladder forward and upward occurs at a much later stage. It is probably brought about through the great increase in size of the internal yolk sac.

11. The minor hepatic ducts arise as the elongated pedicles of definitely placed groups of hepatic tubules. These tubule groups are differentiated at the time when the liver loses its dorsal connection with the fore gut.

12. The variations in position of the minor hepatic ducts in the adult depend upon the degree of shifting of these tubule groups at the time when the main hepatic pouches are differentiated into hepatic ducts and when the rotations mentioned in sections 5 and 8 take place.

13. The gall bladder appears much later than do the primary lateral hepatic diverticula. It arises posterior to the hepatic anlage as a distinct evagination of the gut at the juncture of the floor of the fore gut and anterior wall of the yolk sac, and its intimate connection with the liver duct system is acquired secondarily.

14. The gall bladder loses its dorsal and posterior connection with the gut and is shifted forward and downward until its greatest axis is in the transverse plane of the body. In this way its connection with the ductus choledochus comes to lie in front of that of the lateral hepatic ducts.

15. Subsequently the gall bladder is again shifted upward and forward so that the cystic duct passes backward and downward to join the ductus choledochus. This is brought about by the agency mentioned in section 10.

16. The ductus choledochus is formed from three distinct elements: the anterior part from the pars ductus mediana, a deriva-

tive of the secondary median hepatic pouch; the middle part from the pars ductus lateralis, a derivative of the posterior part of the primary hepatic diverticula, and a posterior part formed from the floor of the duodenum and valvular intestine.

17. Finally, the results of this study may be summarized in tabular form as given below, starting with the sources of the hepatic apparatus on the left hand side, and ending with end results of their evolution on the right. In this table the minor ducts are arranged in the way that they seem to occur most frequently in the late embryo or new-born fish. To this table should be added the statement that all those embryonic structures which are included under the term 'pars hepatica' give rise to hepatic trabeculae as well as to the conducting structures which are listed here.

TABLE 3

Summary of the origin of hepatic structures in Acanthias

Primitive embryonic structures	Intermediate embryonic structures					Fully developed structures	
Mid gut	Duct derived from floor of duodenum and anterior part of valvular intestine					Proximal part	Ductus choledochus
Lateral hepatic diverticula	Pars ductus lateralis					Intermediate part	
	Anterior median pouch	Posterior part (Pars ductus mediana)				Distal part	
		Anterior part (Pars hepatica mediana)	Tubule groups	Right medial		Right medial hepatic ramus	
				Left medial		Left medial hepatic ramus	
			Body	Right side		But slightly developed, may be considered as forming a small part of the distal end of the ductus choledochus	
				Left side		Proximal part	Left hepatic duct
	Lateral hepatic pouches (Pars hepatica lateralis)	Left hepatic pouch	Body			Intermediate part	
			Tubule groups	Posterior left	Ventral cluster	Distal part Minor hepatic rami	
					Dorsal cluster	Dorsal posterior ramus	
				Anterior left		Anterior left ramus	
		Right hepatic pouch	Body			Proximal part	Right hepatic duct
			Tubule groups	Posterior right	Ventral cluster	Distal part Minor hepatic rami	
					Dorsal cluster	Dorsal posterior ramus	
				Anterior right		Anterior right ramus	
Cystic evagination						Cystic duct Gall bladder	

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PLATES

PLATE 1

EXPLANATION OF FIGURES

31 Lateral view of a reconstruction of a part of the archenteron of an Acanthias embryo 6.4 mm. long (S.C. 19). × 100.

32 Anterior view of the reconstruction seen in figure 31. × 100.

33 Lateral view of a reconstruction of the hepatic region of an Acanthias embryo 7.5 mm. long (H.E.C. 1503). × 75.

34 Anterior view of the reconstruction seen in figure 33.

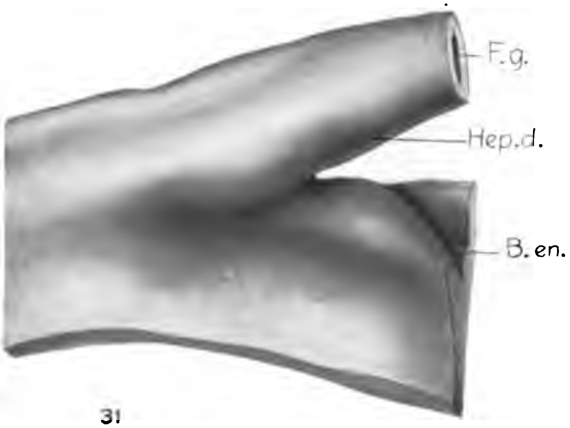
35 Ventral view of a reconstruction of the hepatic region of an Acanthias embryo 9 mm. long (H.E.C. 1495). × 75.

36 Anterior view of the reconstruction seen in figure 35. × 75.

37 Ventral view of a reconstruction of the hepatic region of an Acanthias embryo 7.5 mm. long (S.C. 15). × 75.

38 Anterior view of the reconstruction seen in figure 37. × 75.

B.en., blastodermic entoderm
D.cyst., cystic duct
F.g., fore gut
G.bl., gall bladder
Hep.d., hepatic diverticulum
L.hep.p., lateral hepatic pouch
M.hep.p., median hepatic pouch
T.a., anlagen of hepatic tubules
Y.s., anterior wall of yolk stalk



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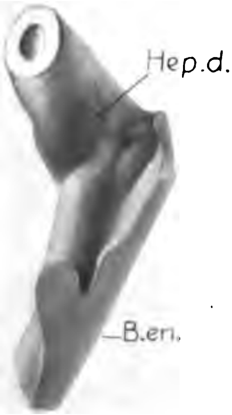

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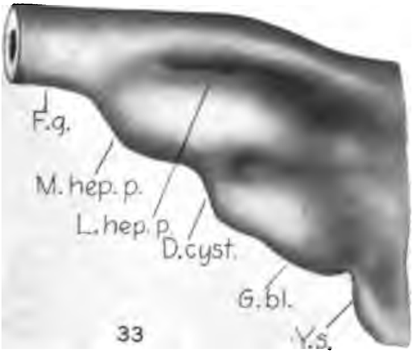

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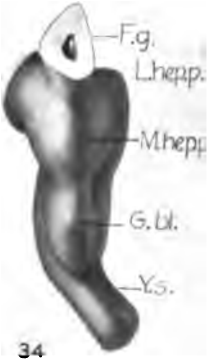

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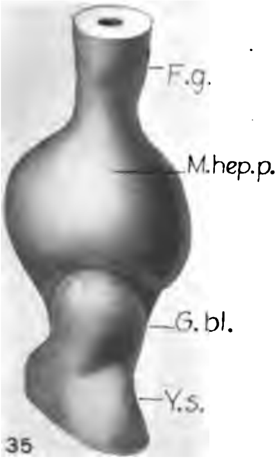

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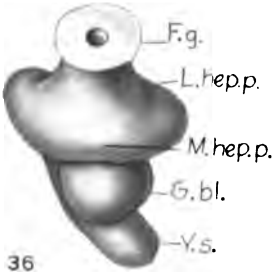

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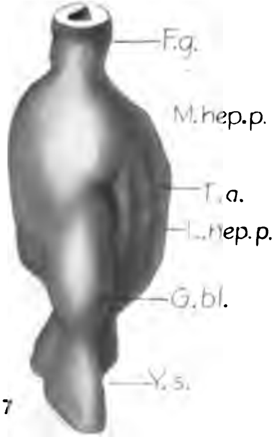

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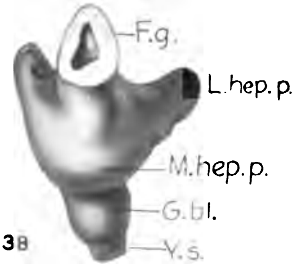


38

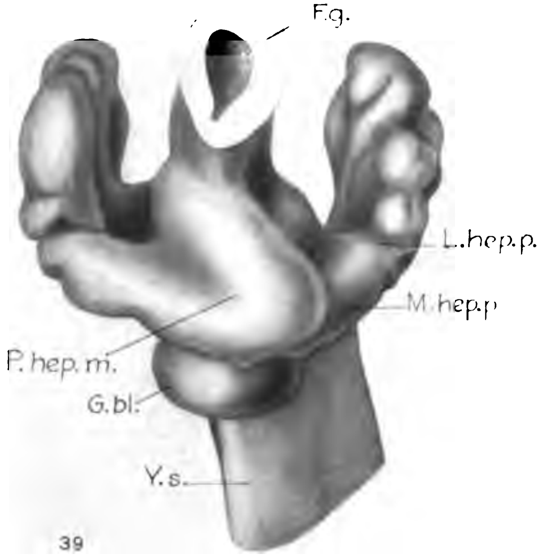
PLATE 2

EXPLANATION OF FIGURES

39 Anterior view of a reconstruction of the liver of an Acanthias embryo 10 mm. long (S.C. 20). × 100.

40 Left lateral view of the same reconstruction. × 100.

F.g., fore gut
G.bl., gall bladder
L.hep.p., lateral hepatic pouch
M.hep.p., median hepatic pouch
P.hep.m., pars hepatica medialis
T.a., anlagen of hepatic tubules
Y.s., yolk stalk



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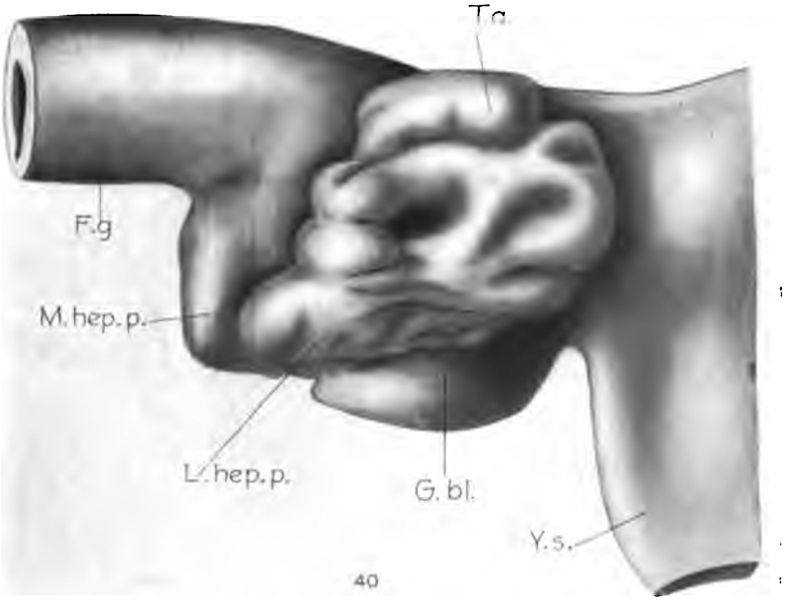

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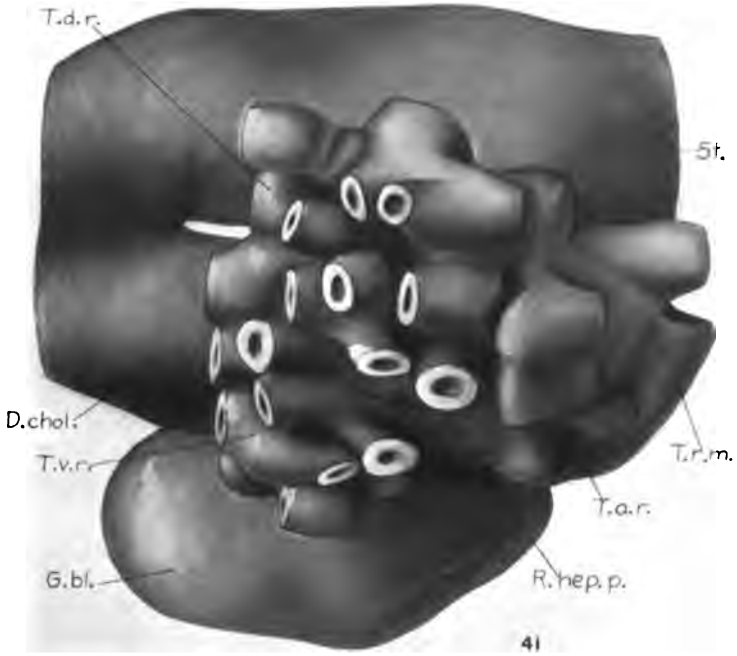
PLATE 3

EXPLANATION OF FIGURES

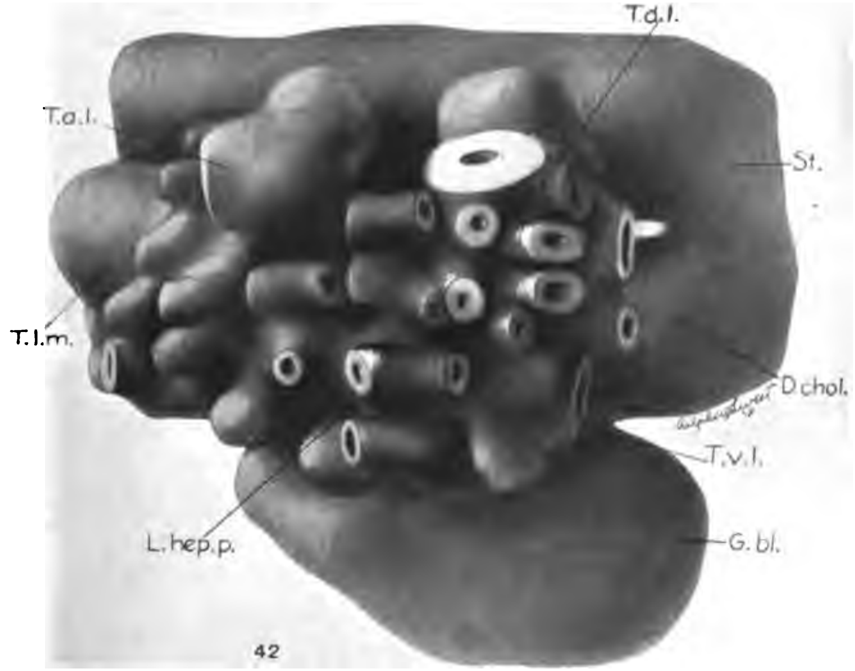
41 Right lateral view of a reconstruction of the liver of an Acanthias embryo 15 mm. long (H.E.C. 227). × 150.

42 Left lateral view of the same reconstruction.

D.chol., ductus choledochus
G.bl., gall bladder
L.hep.p., left hepatic pouch
R.hep.p., right hepatic pouch
St., stomach
T.a.l., anterior left tubule group
T.a.r., anterior right tubule group
T.l.m., left medial tubule group
T.d.l., dorsal cluster, posterior left tubule group
T.v.l., ventral cluster, posterior left tubule group
T.r.m., right medial tubule group
T.d.r., dorsal cluster, posterior right tubule group
T.v.r., ventral cluster, posterior right tubule group


T.d.r.
St.
D.chol.
T.v.r.
T.r.m.
T.a.r.
G.bl.
R.hep.p.

41


T.d.l.
T.a.l.
St.
T.l.m.
D.chol.
T.v.l.
L.hep.p.
G.bl.

42

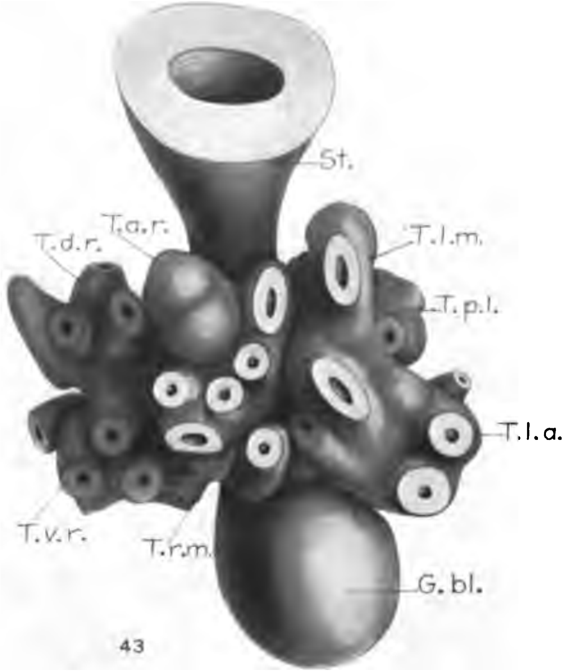
PLATE 4

EXPLANATION OF FIGURES

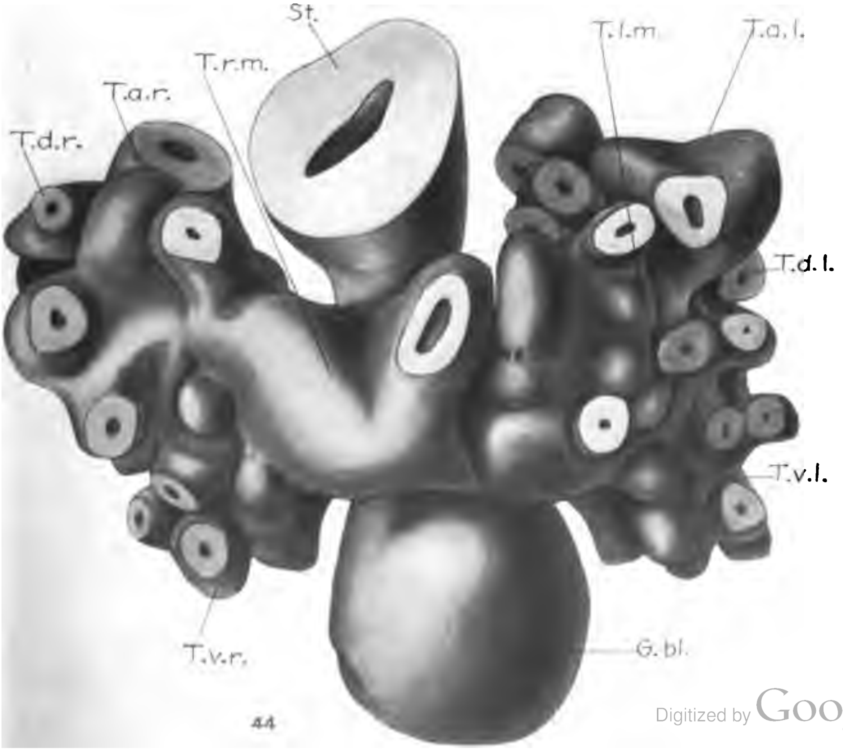
43 Anterior view of a reconstruction of the liver of an Acanthias embryo 15.5 mm. long (S.C. 1). × 120.

44 Anterior view of the reconstruction illustrated in figures 41–42. × 150.

G.bl., gall bladder
St., stomach
T.a.l., *T.l.a.*, anterior left tubule group
T.a.r., anterior right tubule group
T.d.l., dorsal cluster, posterior left tubule group
T.d.r., dorsal cluster, posterior right tubule group
T.l.m., left medial tubule group
T.p.l., posterior left tubule group—the separate clusters cannot be seen in this view of figure 43.
T.r.m., right medial tubule group
T.v.l., ventral cluster, posterior left tubule group
T.v.r., ventral cluster, posterior right tubule group


St.
T.a.r.
T.d.r.
T.l.m.
T.p.l.
T.l.a.
T.v.r.
T.r.m.
G.bl.

43


St.
T.r.m.
T.a.r.
T.d.r.
T.l.m.
T.a.l.
T.d.l.
T.v.l.
T.v.r.
G.bl.

44

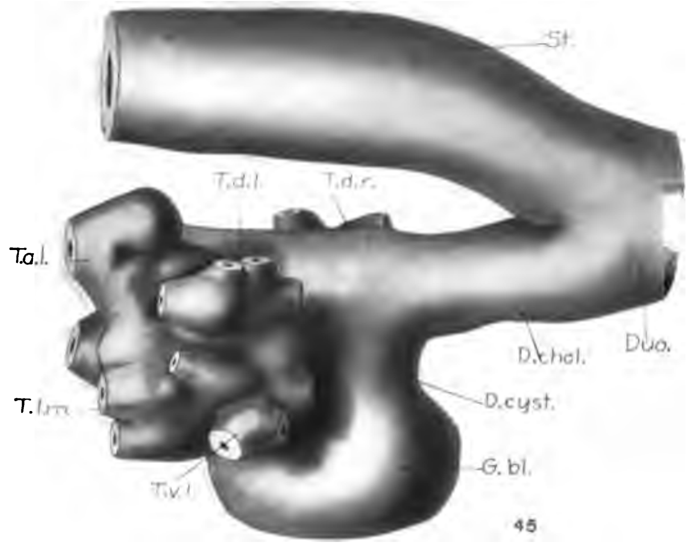
PLATE 5

EXPLANATION OF FIGURES

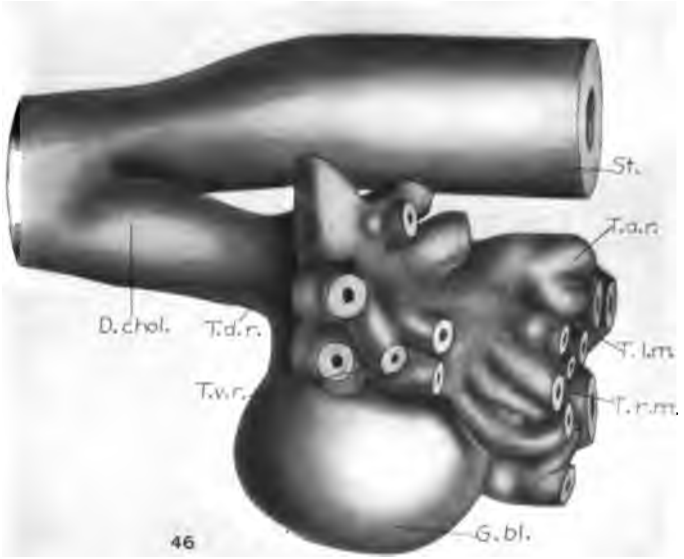
45 Left lateral view of a reconstruction of the liver of an Acanthias embryo 15.5 mm. long (S.C. 1). × 100.

46 Right lateral view of a reconstruction of the same reconstruction. × 100.

D.chol., ductus choledochus
D.cyst., cystic duct
Duo., duodenum
G.bl., gall bladder
St., stomach
T.a.l., anterior left tubule group
T.a.r., anterior right tubule group
T.d.l., dorsal cluster, posterior left tubule group
T.d.r., dorsal cluster, posterior right tubule group
T.l.m., left medial tubule group
T.r.m., right medial tubule group
T.v.l., ventral cluster, posterior left tubule group
T.v.r., ventral cluster, posterior right tubule group


St.
T.d.l.
T.d.r.
T.a.l.
D.chol.
Duo.
D.cyst.
T.l.m.
G.bl.
T.v.l.

45


St.
T.a.r.
D.chol.
T.d.r.
T.l.m.
T.v.r.
T.r.m.
G.bl.

46

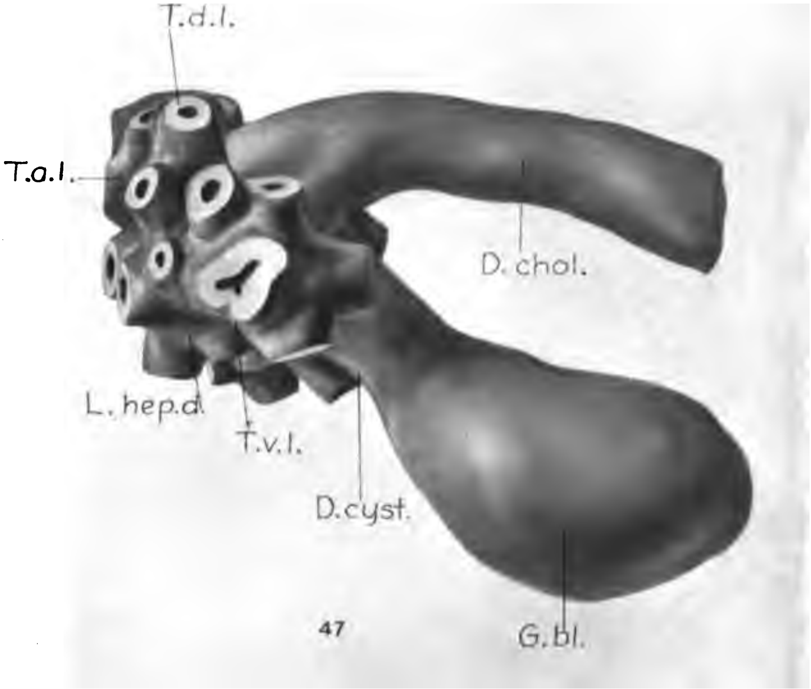
PLATE 6

EXPLANATION OF FIGURES

47 Left lateral view of a reconstruction of the liver of an Acanthias embryo 20.5 mm. long (S.C. 5). × 75.

48 Right lateral view of the same object. × 75.

D.chol., ductus choledochus
D.cyst., cystic duct
G.bl., gall bladder
L.hep.d., left hepatic duct
T.a.l., anterior left tubule group
T.a.r., anterior right tubule group
T.d.l., dorsal cluster, posterior left tubule group
T.d.r., dorsal cluster, posterior right tubule group
T.v.l., ventral cluster, posterior left tubule group
T.v.r., ventral cluster, posterior right tubule group



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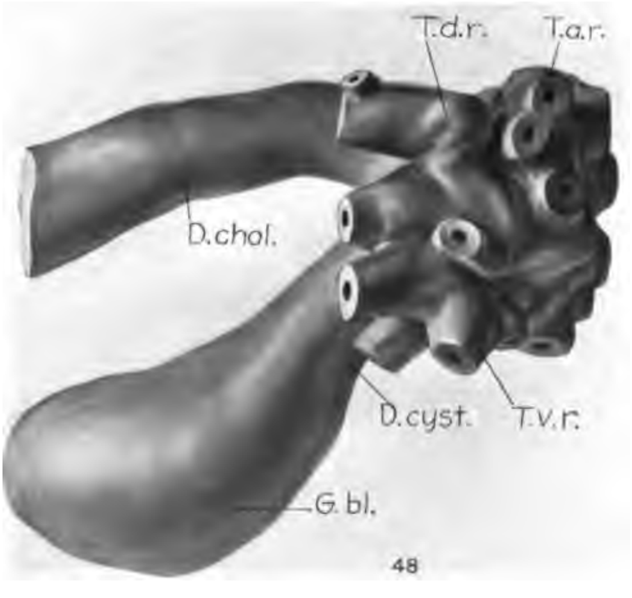

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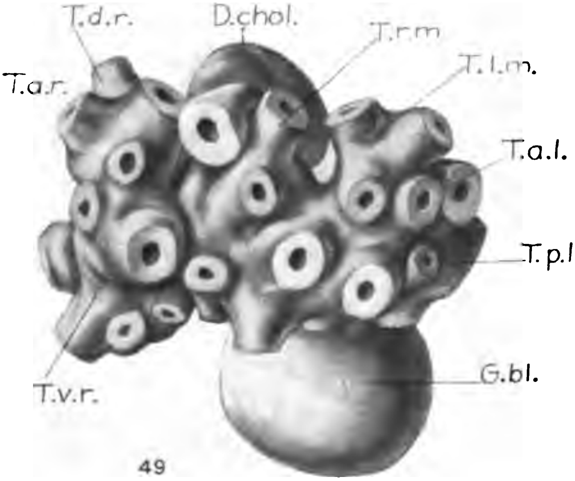
PLATE 7

EXPLANATION OF FIGURES

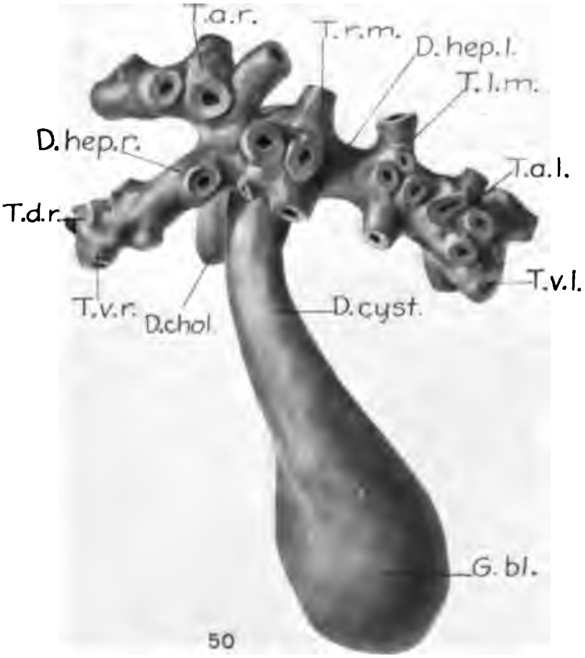
49 Anterior view of the reconstruction shown in figures 47 and 48. × 75.

50 Anterior view of a reconstruction of the gall bladder and liver ducts of an Acanthias embryo 20.6 mm. long (H.E.C. 1494). × 75.

D.chol., ductus choledochus
D.cyst., cystic duct
D.hep.l., left hepatic duct
D.hep.r., right hepatic duct
G.bl., gall bladder
T.a.l., anterior left hepatic ramus or tubule group
T.a.r., anterior right ramus or tubule group
T.p.l., dorsal cluster, posterior left tubule group, which later forms the posterior left ramus
T.d.r., dorsal cluster, posterior right tubule group
T.l.m., left medial ramus or tubule group
T.r.m., right medial ramus or tubule group
T.v.l., minor tubules of ventral left tubule group
T.v.r., minor tubules of ventral right tubule group


T.d.r.
D.chol.
T.r.m
T.a.r.
T.l.m.
T.a.l.
T.p.l.
T.v.r.
G.bl.

49


T.a.r.
T.r.m.
D.hep.l.
T.l.m.
D.hep.r.
T.a.l.
T.d.r.
T.v.l.
T.v.r.
D.chol.
D.cyst.
G.bl.

50

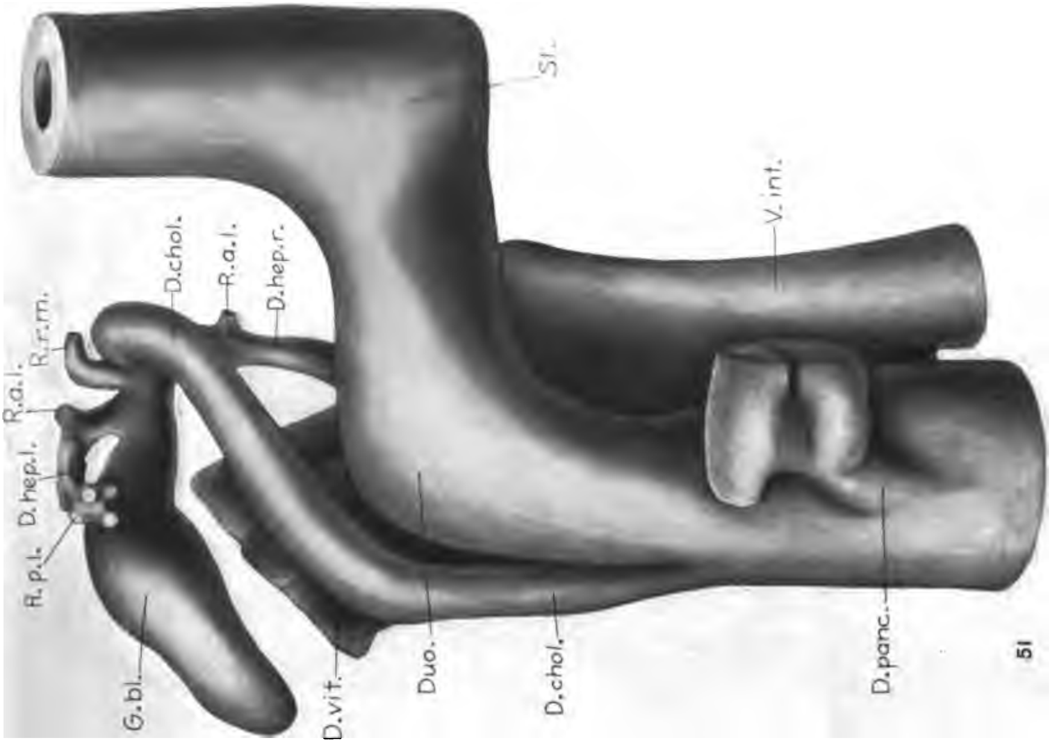
PLATE 8

EXPLANATION OF FIGURES

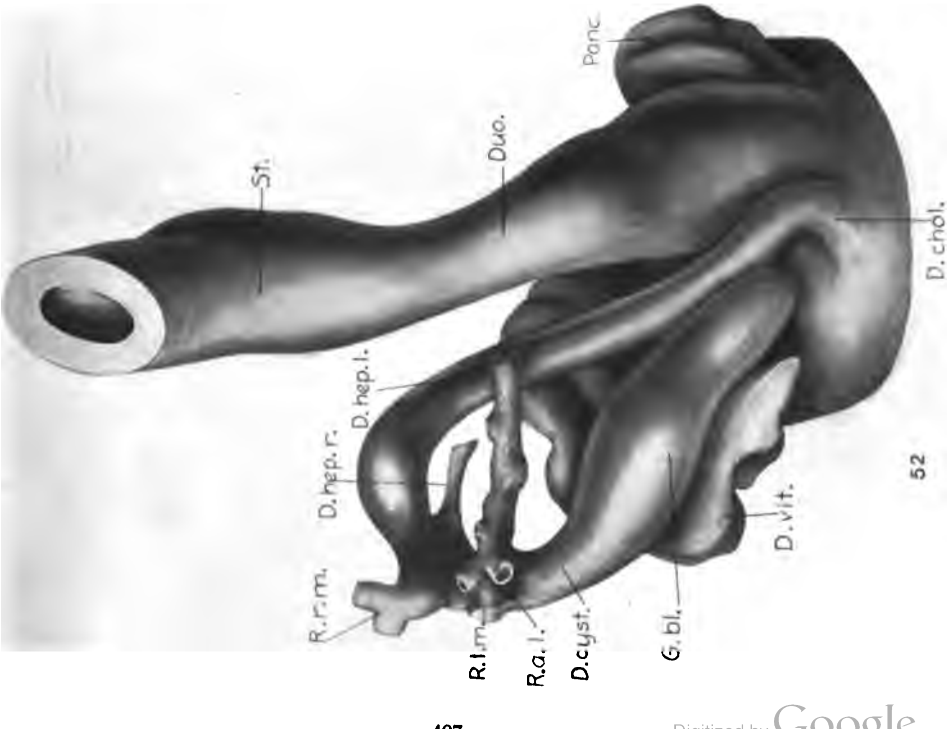
51 Left lateral view of a reconstruction of a part of the gut and the liver ducts and gall bladder of an Acanthias embryo 28 mm. long (S.C. 6). × 50.

52 Antero-ventral view of the same reconstruction. × 50.

D.chol., ductus choledochus
D.cyst., cystic duct.
D.hep.l., left hepatic duct
D.hep.r., right hepatic duct
D.panc., pancreatic duct
D.vit., vitelline duct
Duo., duodenum
G.bl., gall bladder
Panc., pancreas
R.a.l., anterior left hepatic ramus
R.a.r., anterior right ramus
R.l.m., left medial ramus
R.p.l., posterior left ramus
R.r.m., right medial ramus
St., stomach
V.int., valvular intestine


St.
V. int.
D.chol.
R.a.l.
D.hep.r.
R.r.m.
R.a.l.
D.hep.l.
R.p.l.
G.bl.
D.vit.
Duo.
D.chol.
D.panc.

51


Panc.
Duo.
St.
D.chol.
D.hep.l.
D.hep.r.
R.r.m.
R.l.m.
R.a.l.
D.cyst.
G.bl.
D.vit.

52

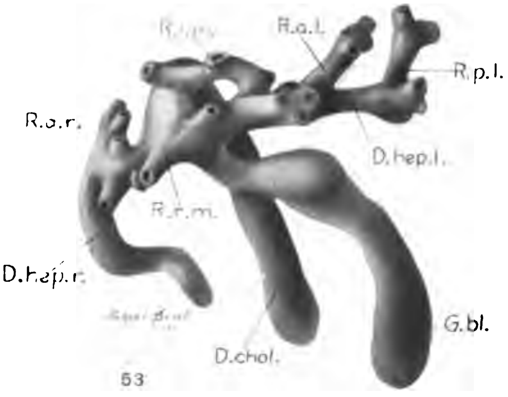
PLATE 9

EXPLANATION OF FIGURES

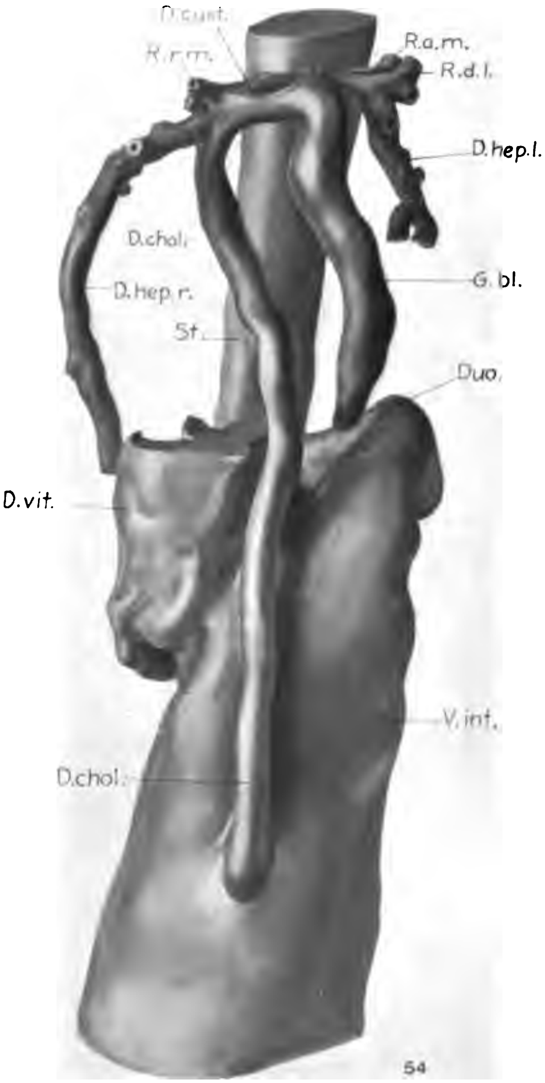
53 Anterior view of a reconstruction of the gall bladder and liver ducts of an Acanthias embryo 33.1 mm. long (S.C. 8). × 50.

54 Ventral view of a reconstruction of the gall bladder, liver ducts, and a part of the gut of the same embryo. × 50.

D.chol., ductus choledochus
D.cyst., cystic duct
D.hep.l., left hepatic duct
D.hep.r., right hepatic duct
D.vit., vitelline duct
Duo., duodenum
G.bl., gall bladder
R.a.l.,*R.a.m.*,anterior left hepatic ramus
R.a.r., anterior right ramus
R.d.l., dorsal posterior left ramus
R.l.m., left medial ramus
R.p.l., posterior left ramus
R.r.m., right medial ramus
St., stomach
V.int., valvular intestine


R.a.l.
R.p.l.
R.a.r.
D.hep.l.
R.r.m.
D.hep.r.
G.bl.
D.chol.

53


D.cyst.
R.r.m.
R.a.m.
R.d.l.
D.hep.l.
D.chol.
G.bl.
D.hep.r.
St.
Duo.
D.vit.
V.int.
D.chol.

54

Reprinted from The Journal of Comparative Neurology, Vol. 23, No. 5
October, 1913

THE MORPHOLOGY OF THE SEPTUM, HIPPOCAMPUS, AND PALLIAL COMMISSURES IN REPLILES AND MAMMALS[1]

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NINETY-THREE FIGURES

In the mammalian brain the hippocampus extends from the base of the olfactory peduncle over the corpus callosum and bends down into the temporal lobe. Over the corpus callosum there is a well developed hippocampus in monotremes and marsupials, while in higher mammals it is reduced to a slender vestige consisting of the stria longitudinalis and indusium. The telencephalic commissures in monotremes and marsupials form two transverse bundles in the rostral wall of the third ventricle. We owe our knowledge of the history of the pallial commissures in mammals chiefly to the work of Elliot Smith. This author states that these commissures are both contained in the lamina terminalis. The upper (dorsal) commissure represents the commissure hippocampi or psalterium; the lower (ventral) contains the commissura anterior and the fibers which serve the functions of the corpus callosum. In mammals as the general pallium grows in extent there is a corresponding increase in the number of corpus callosum fibers. These fibers are transferred from the lower to the upper bundle in the lamina terminalis, in which corpus callosum and psalterium then lie side by side. As the pallium grows the corpus callosum becomes larger, rises up and bends on itself until it finally forms the great arched structure which we know in higher mammals and man.

During all this process two changes have taken place in the lamina terminalis. First, it was invaded by cells from the neighboring medial portion of the olfactory lobe so that the paired medial olfactory nuclei came to be connected by a median mass through which the commissures crossed from side to side. The median mass was called by Elliot Smith the commissure bed and the whole body, including the paired gray masses, was called the paraterminal body. Second, the enlargement and arching up of the corpus callosum caused the stretching of the lamina terminalis and the commissure bed. The space within the concavity of the callosal arch was filled by a part of the paraterminal body. This part of the medial wall is thick in lower forms but the great arching of the corpus callosum in higher mammals has stretched it out into the thin septum pellucidum.

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[1] Neurological studies, University of Minnesota, no. 18.

This very clear conception of the commissures and their relationships has been generally accepted and has been followed by the writer. Elliot Smith and others have carried this conception into the study of reptiles and have based upon it some homologies in the brains of amphibians and fishes. The writer has approached all questions of brain morphology from a different standpoint. He has directed his attention always to the more primitive forms, making the attempt first to see the structure and relationships existing in those brains, with the hope that thereafter it would be possible to see with certainty how the specialized brains of higher forms have been developed from the lower. The method heretofore followed by most workers has been to study man and mammals first, as the animals in which we are directly interested, and then to apply the results to lower vertebrates either as a matter of curiosity or as a means of securing corroborative evidence of their accuracy. The genetic method is more tedious but if followed with caution and fidelity to the actual facts it should lead to permanent results. Caution is necessary in the forms chosen for study and the significance accorded to the facts discovered. If we are to use lower forms to throw light upon the human brain, those forms must be chosen for study which are at once the least specialized and most nearly allied to the ancestors of mammals and man. In studying such brains attention must be given to the habits of the animals and the consequent degree of development of each system of nerve centers.

The work of the writer has shown that the telencephalon of cyclostomes is truly primitive as compared with that of other fishes. It is believed that the more primitive selachians are the nearest existing allies to the common ancestors of fishes, amphibians and reptiles. Among the reptiles those which are regarded by paleontologists as nearest the line of mammalian descent are the chelonia.

The writer's reasoning has been that an understanding of the morphology of the mammalian and human brain must rest on a clear knowledge of the steps in its evolution and of the forces (habits, mechanical factors, etc.) which have been concerned in that evolution. For this purpose we must be able to give a consistent account of the evolution of the brain through the chief groups of vertebrates which stand nearest the line of descent of higher mammals: the cyclostomes, selachians, dipnoi, chelonia, monotremes, marsupials, and certain mammals. With this in view the writer, having studied the telencephalon in cyclostomes and selachians, wishes to present here some contributions upon reptiles and mammals. Further studies of dipnoi, as affording a bridge between selachians and reptiles, are greatly to be desired.

The reason for the above statement of the writer's method of approach to this problem is that the facts thus far observed in the telencephalon of primitive vertebrates do not agree with the accepted views regarding the mammalian brain. The difficulties have to do chiefly with the relations of the paraterminal body, lamina terminalis, commissures and hippocampus. In selachians the pallial commissures are imbedded in a massive roof (called by the writer the primordium hippocampi) and cross through the lamina supraneuroporica, wholly independent of the lamina terminalis and the paraterminal body. The fiber connections of the body called primordium hippocampi are so characteristic and the line of demarcation between it and the paraterminal body is so clear, that there can be no question of the individuality of the primordium hippocampi in selachians and cyclostomes. The only doubt which has arisen in the use of this term has been whether the body in question may not give rise to something more than the hippocampal formation in the mammalian brain (Johnston '10 c, p. 149). With the study of the brains of a number of reptiles and mammals, the conviction has gradually forced itself on the writer that the prevailing ideas regarding the morphology of the medial wall of the telencephalon in mammals must be modified in view of the fundamental relations in lower vertebrates. The evidence for this can best be presented by careful comparison of the chief structures in this region in lower and higher vertebrates.

For the sake of clearness it must be understood that the term primordium hippocampi is used in the sense in which the writer has employed it in his previous papers. The fitness of this and other terms is discussed in a section at the end of this paper.

THE ROOF-PLATE IN THE TELENCEPHALON

The anterior end of the brain floor is occupied by the optic chiasma ('09 b). The roof-plate begins at the preoptic recess and extends in a curve around the topographic rostral end of the brain and caudad into the roof of the diencephalon ('10 c). The velum transversum, always present in vertebrates at least in embryos, marks the boundary between diencephalon and telencephalon in the roof ('09 b). The telencephalic roof-plate consists of three portions, the lamina terminalis, lamina supraneuroporica and tela chorioidea ('11 b, p. 491). The lamina terminalis is formed by the fusion of the lips of the primitive neuropore and is bounded above by the neuroporic recess which marks the point of latest connection of the neural tube with the ectoderm. The lamina supraneuroporica is thickened by commissural fibers in cyclostomes and by gray matter and fibers in selachians, and even in ganoids and bony fishes where it usually contains no commissures it is thickened and is histologically different from the tela chorioidea ('11 b). From the tela chorioidea just rostral to the velum transversum arises the paraphysis. The attachment of the tela chorioidea to the dorsal border of the wall of the telencephalon medium is known as the taenia fornicis ('11 a, p. 6). The taeniae of the two sides converge rostrally to meet at the point of junction of the tela chorioidea with the lamina supraneuroporica in the mid-line. This point has been labelled *m* in numerous figures in preceding papers. Since there is an early differentiation between the tela chorioidea and the lamina supraneuroporica in the embryo, this point is always easily recognizable in both embryos and adults of all vertebrates. It is one of the fundamental landmarks in the telencephalon. Its position has been noted by Elliot Smith ('97 b, p. 52, fig. 23; '97 c, p. 235) and is indicated in figures 4, 5, 6, 7, 9, 35, 45, 55, 64 of this paper. This point may be named *margo posterior pallii.*

To complete the identification of lamina terminalis and lamina supraneuroporica in reptiles and mammals it is necessary only to determine the position of the recessus neuroporicus.

The neuroporic recess is always situated rostral or dorsal to the anterior commissure. Dorsal to the recess in cyclostomes and selachians is the lamina supraneuroporica with its commissures and gray matter as already mentioned. In selachians there is found an external neuroporic recess ('11 a) in the form of a deep pit or a slender canal, through which blood vessels enter the brain near the upper border of the lamina terminalis.

In all the embryos of reptiles and mammals which the writer has examined there is found a ventricular recess rostral to the anterior commissure, and above the recess is a thick lamina which in advanced embryos contains a pallial commissure. These relations were shown in a previous paper ('10 c, figs. 17, 18, 19, 20). It will be seen at once upon comparison with selachian embryos that the relations are essentially the same. This recess is not preserved in the adults of all reptiles and mammals, but its position is readily determined, since it must lie between the anterior and the pallial commissures. The recess which is found in this position is the recessus triangularis of Schwalbe ('81). Its significance will be discussed in the following paragraphs.

The location of the recessus neuroporicus is of critical importance. Most authors who have recognized a neuroporic recess in mammals have identified it with the angulus terminalis of His and the recessus superior, the relations of which are so clearly figured by Elliot Smith in monotreme, marsupial and mammalian brains.

His in his Allgemeine Morphologie in 1892 (p. 349, 352) defined the lamina terminalis as the frontal portion of the frontal seam of closure. Below, the lamina terminalis ends in the recessus opticus (rec. praeopticus) and is followed by a basal portion of the frontal seam containing the optic chiasma. The upper border of the lamina terminalis in human embryos is marked by an inward fold (i.e., the velum transversum) in early stages and later by the beginning of the choroid plexus. Within the lamina terminalis as thus defined (cf. p. 349 and 352) in many animals lies the neuropore.

Von Kupffer ('93) described the neuropore in the sturgeon under the name of lobus olfactorius impar. Von Kupffer's material was unfortunate because the eversion of the pallial part of the telencephalon in ganoids leaves the recessus neuroporicus apparently bounded dorsally by a non-nervous membrane only. Comparison of v. Kupffer's figure 18 with the writer's figure of this region in the adult sturgeon brain ('11 b, fig. 52) will show that there intervenes between the locus of the recessus neuroporicus and the plexus chorioideus a membrane which is distinctly thicker than the choroid plexus and differs from it histologically. v. Kupffer ('94) also described the recessus neuroporicus in Petromyzon and it has since been shown by Sterzi ('07) that this lies below the dorsal telencephalic commissure. Upon direct comparison of the adult human brain with that of the sturgeon embryo, v. Kupffer believed (p. 39) the recessus triangularis of Schwalbe ('81) to be the homologue of his lobus olfactorius impar.

In the comments made by His ('93) upon v. Kupffer's work, he identified v. Kupffer's lobus olfactorius impar with the angulus terminalis of His. This angulus terminalis is the meeting point of the tela chorioidea with the dorsal border of the lamina terminalis as defined in 1892. This does not agree with v. Kupffer's view cited above, nor does it agree with His's own statement in 1892 that the neuropore lies within the lamina terminalis.

Burckhardt ('94 b) identified v. Kupffer's lobus olfactorius impar with a recess in reptiles situated at the point of junction of the choroid plexus with the lamina containing the dorsal commissure. To this recess Burckhardt gave the name recessus neuroporicus and to the ependymal membrane which stretches from this recess to the paraphysis he gave the name lamina supraneuroporica. Burckhardt ('94 c) then compared this ependymal lamina supraneuroporica with the tela chorioidea of various fishes, implying that the neuroporic recess was located at the point of attachment of the tela to the massive roof in front. In all this Burckhardt was in agreement with His 1893. In his last work, however, Burckhardt ('07) clearly described the neuroporic recess in Scymnus and shows that in selachians the lamina supraneuroporica is a true nervous structure. It is in this sense that the term lamina supraneuroporica must be used and not in the sense in which Burckhardt first used it.

In the meantime Elliot Smith ('96 a, '99) following Burckhardt, identified the recessus superior of mammalian brains with the angulus terminalis of His and with the recessus neuroporicus. There can be no doubt that the recessus superior is the same as the angulus terminalis (figs. 8, 9, *m*) and that it corresponds to the recess which Burckhardt called neuroporic recess in reptiles. In both classes this recess lies just over the dorsal telencephalic commissure and is bounded above by the tela chorioidea, as clearly figured by Elliot Smith. That it does not correspond to what is known to be the neuroporic recess of selachians is obvious, since in the latter case the neuroporic recess is separated from the tela chorioidea by a thickened lamina containing the dorsal commissures.

The chief contribution by v. Kupffer in this connection was his pointing out the importance of the neuroporic recess as a landmark in forebrain morphology. His in 1892 presented a clear conception of the neural tube as closed rostrally by the lamina terminalis within whose extent the neuropore appeared in some animals. In 1893 he inadvertently abandoned this definition by placing the neuropore at the dorsal border of the lamina terminalis where the latter meets the choroid plexus. Since then some authors have defined the lamina terminalis as *the membrane formed by the closing of the neuropore and bounded* *dorsally by the neuroporic recess.* Other authors have defined the lamina terminalis as *the closing membrane which is bounded above by the choroid plexus.* Both groups of workers believe that they are following His. The result has been the greatest confusion. In some cases even the tela chorioidea has been included in the lamina terminalis. The writer holds the view that the lamina terminalis is coextensive with the primitive neuropore and is bounded above by the neuroporic recess, but insists that the locus of this recess shall be accurately determined. It will appear below that His was in error in locating the neuroporic recess at his angulus terminalis.

Conclusive evidence as to the location of the neuroporic recess in reptiles and mammals is to be obtained only by following that recess from the open neuropore through successive stages of development until its relation to the telencephalic commissures is established. The accompanying figures 1 to 6 show the contour of the dorsal seam of the forebrain in embryos of Chelydra serpentina from the stage of the open neuropore to a stage possessing a carapace 8 mm. in length. For the opportunity to study this material the writer is indebted to Dr. C. E. Johnson. The locus of the neuroporic recess is entirely clear throughout these stages. In the latest stage drawn (fig. 6) there appears between the preoptic recess below and the beginning of the tela chorioidea above (*m*) a somewhat S-shaped thickening of the roof-plate. In the lower part of this thickening the fibers of the anterior commissure are present. Above this the neuroporic recess appears as a ventricular pit. The upper part of the thickening is the lamina supraneuroporica.

The same relations are seen in figure 7 which shows the outline of the median section of the brain of a slightly more advanced embryo of Emys lutaria. For the opportunity of studying this embryo I am indebted to Dr. G. Carl Huber. In this the recessus neuroporicus separates widely the lamina supraneuroporica from the thickening in the lamina terminalis which contains the anterior commissure. The lighter outlines numbered *1*, *2*, *3*, *4*, are the outlines of successive parasagittal sections of the medial wall of the right hemisphere. The contours *1* and

2 show that an oblique ridge runs upward and forward from the lamina supraneuroporica into the medial wall of the hemisphere. In the brain of the adult turtle (fig. 9) this ridge is occupied by the hippocampal commissure. The recessus neuroporicus is evident in the adult upon the inner surface of the transverse ridge which is occupied by the anterior and the hippocampal commissures (fig. 9). In Chelydra serpentina the two commissures form a ridge projecting farther into the ventricle and the neuroporic recess is nearly obliterated.

Elliot Smith's ('03) figures and clear description of Hydrosaurus show that a deep recess (his recessus inferior) exists between the anterior and hippocampal commissures in this Monitor. Professor Smith points out that this recess corresponds to the recessus triangularis of Schwalbe in mammalian brains.

In his work on the pineal region of Sphenodon, Dendy ('10) shows in text-figure 1 a median section which agrees very well with my latest stage of Chelydra serpentina. In agreement with Burckhardt's earlier work he applies the name lamina supraneuroporica to the tela chorioidea, while to the thick lamina to which the tela is attached he gives the name cerebral hemisphere. Although of course the hemisphere can not really be seen in a median section, the application of this name by Dendy serves to show that the structure which the writer has called lamina supraneuroporica is a nervous bridge connecting the two hemispheres in Sphenodon as in the turtles.

The same conformation in the region of the anterior and hippocampal commissures in a late embryo of Anguis fragilis is shown in v. Kupffer's figure 248 in his article in Hertwig's Handbuch.

From his own observations on turtles and from the comparison of the results of other workers, the writer is convinced that the neuroporic recess in reptiles is located immediately above the anterior commissure and that the hippocampal commissure crosses the middle line in a lamina supraneuroporica which serves as a massive bridge between the medial walls of the two hemispheres. In these relations the reptiles agree strictly with the selachians.

We have fairly clear and complete evidence as to the location of the neuroporic recess in mammals. In a previous communication ('09 b) the writer identified as this recess a pit located in the pig of 15 mm. and larger just above the anterior commissure. Figure 39 of that paper may be consulted in this connection. The development of the pig has since been carefully reviewed and the writer is convinced of the correctness of this view. The pit in question is the embryonic representative of the recessus triangularis of Schwalbe and lies over the anterior commissure and between the fornix columns. In a later communication ('10c) were given median sections of rabbit and cat embryos showing the same recess between the anterior and the pallial commissures. Sheep embryos show the same relations. Figures 8 and 24 show the neuroporic recess and lamina supraneuroporica in a human embryo of 37 mm., for the opportunity to study which I am indebted to Dr. G. Carl Huber.

Neumayer ('99) figures early stages of rabbit and sheep embryos in which he clearly identifies the neuroporic recess (under the name of lobus olfactorius impar) and shows that it comes to lie between the anterior and pallial commissures. Several of his figures are reproduced in Ziehen's article on the morphogenesis of the central nervous system of mammals in Hertwig's Handbuch. The pit which the writer identified as neuroporic recess in cat embryos was figured by Martin ('93) who showed that the so-called lamina terminalis grows thinner in later stages at the location of this pit. It is well known that the recessus triangularis (rec. inferior of Elliot Smith) exists in this same position in adult monotremes, marsupials and many mammals. Werkman ('13) in his recent work on the development of the commissures in lower mammals shows the neuroporic recess in embryos of the mole and the bat (figs. 5, 8, 13, 15, 17, 18, *c.ep.*).

It is proposed that hereafter the name recessus neuroporicus be adopted for this pit in the brains of all vertebrates, instead of recessus triangularis (Schwalbe) or recessus inferior (Elliot Smith). The recessus neuroporicus may be defined as a pit existing in the embryos or adults of all vertebrates in the topographic rostral wall of the third ventricle, marking the point at which the dorsal seam of closure of the neural tube last separated form the ectoderm. It lies always topographically dorsal (or rostral) to the anterior commissure and in all forms which possess a true fornix and pallial commissures it lies beneath the pallial commissures and between the columns of the fornix.

The lamina terminalis is the anterior portion of the roof-plate and is formed by the fusion of the lips of the anterior neuropore. It extends from the anterior border of the chiasma ridge to the neuroporic recess and is traversed by the anterior commissure. The lamina supraneuroporica is a thickened portion of the roof-plate extending dorso-caudad from the neuroporic recess and containing pallial commissures in cyclostomes, selachians, some ganoids and teleosts, dipnoi, reptiles and mammals. The tela chorioidea telencephali is the anterior part of the membranous roof of the third ventricle, attached rostrad to the lamina supraneuroporica, containing within its extent the paraphysis, and separated from the tela chorioidea diencephali by the velum transversum. The evidence on which these definitions rest has been examined by the writer personally in all classes of vertebrates except the Dipnoi. For information regarding the position of the pallial commissures in Dipnoi I am indebted to Prof. G. Elliot Smith who states in a letter that the pallial commissure in Lepidosiren is disposed as in reptiles.

GROSS RELATIONS IN THE MEDIAL WALL OF THE HEMISPHERE

In evaginated brains such as those of the selachians, amphibians, reptiles and mammals the hemisphere projects rostrad beyond the lamina terminalis and possesses a medial wall. Between the apposed medial walls of the two hemispheres is the great sagittal fissure which is closed caudally by the lamina terminalis and lamina supraneuroporica. The lower part of the medial wall (olfactory lobe) undergoes a secondary fusion in most selachains. In reptiles and mammals there is only a thickening of the lamina terminalis and of the lamina supraneuroporica related to the anterior and pallial commissures. In selachians these thickenings and the secondary fusions become so extensive that the external neuroporic recess is reduced to a slender canal. Along the line of this canal the medial wall presents a cell-free zone reaching from the vicinity of the neuroporic recess around the rostral end of the hemisphere into the lateral wall. This cell-free area which the writer has called the zona limitans hippocampi, marks the limit between secondary olfactory centers below and the olfacto-gustatory correlating center above, the primordium hippocampi.

In the comparison of the telencephalon of selachians with that of reptiles and mammals the identification of this zona limitans would be of the greatest convenience. It may be said at once that this boundary line is not always easy to recognize. A cell-free zone occurring in the medial wall of the hemisphere is not necessarily homologous with the zona limitans hippocampi of selachians. The line of demarcation between any two functionally differentiated nuclei often presents itself in the form of a cell-free zone. It is therefore necessary to examine the relations of the centers in question. In all vertebrates the secondary olfactory centers receive olfactory tract fibers and send fibers to the hypothalamus and to the nucleus habenulae. In selachians, if we confine our attention to the medial wall of the hemisphere, three kinds of fibers pass across the zona limitans between the medial olfactory nucleus and the hippocampal primordium: (a) olfactory tract fibers, (b) fibers arising from the cells of the medial olfactory nucleus and tuberculum olfactorium, known as the tractus olfacto-corticalis septi, and (c) the fornix columns which descend from the hippocampal primordium to pass caudad. In selachians the primordium hippocampi presents no arrangement of cells into layers. The medial olfactory nucleus presents two poorly marked cell layers. The outer one is continuous below with the more compact and definite cortical layer of the tuberculum olfactorium. This nucleus is present in reptiles and mammals but has received little attention from previous authors. It is shown in Herrick's figures 46 and 66 where it is called nucleus medianus septi; but the nucleus so named in figure 43 is the primordium hippocampi and the nucleus so named in figure 47 is that part of the paraterminal body which surrounds the recessus praeopticus. The deeper nucleus forms a projection into the lateral ventricle and has been called nucleus accumbens septi (Kappers and Theunissen) nucleus septi (Unger) and nucleus lateralis septi (Herrick). I shall hereafter speak of the medial olfactory area as the area parolfactoria and shall use for the two nuclei contained in it the names nucleus parolfactorius medialis and lateralis, respectively. The general features of the relations in the medial wall in selachians are shown in figure 10.

When the medial wall of a reptilian brain is compared with that of the selachian there seems at first sight no great difficulty in identifying the hippocampal formation and the area parolfactoria. The hippocampus has been identified with the mediodorsal cortex by Meyer ('92), Elliot Smith ('96, '10), Levi ('04) and others. Below the rostral end of the hippocampal formation the area parolfactoria is thickened so as to form a ridge in the ventricle. This is the anterior end of the paraterminal body of Elliot Smith and according to the account given by that author ('03) this body becomes united with its fellow through the lamina terminalis, thus forming the bed of the anterior and hippocampal commissures. The paraterminal body also extends caudad in the medial wall of the hemisphere over the interventricular foramen. In certain forms this supraforaminal portion of the paraterminal body continues to the caudal pole where it again unites with its fellow (e.g., in Sphenodon) to form the bed of the commissura aberrans. In mammals it is stated that this paraterminal body surrounds and embeds the entire system of hemispheral commissures (c. anterior, c. hippocampi and corpus callosum). These interpretations are diagrammatically represented in figures 82 and 83.

The problem before us is to determine what parts of the medial wall of the hemisphere in reptiles and mammals have been derived from that definite morphological and physiological unit in selachians which the writer has called the primordium hippocampi, and what parts have been derived from the area parolfactoria. The examination which follows will show that the paraterminal body of Elliot Smith includes parts of both the area parolfactoria and the primordium hippocampi of selachians and that the bed of the pallial commissures is of different origin and has different morphological relations from the bed of the anterior commissure.

In figures 14 and 17 are shown two transverse sections of the telencephalon of the hinge turtle (Cistudo carolina), one near the olfactory peduncle and one near the interventricular foramen. In the section near the foramen the locus of the recessus neuroporicus is indicated by the position of the anterior commissure. If we compare in the turtle and the selachian that portion of the hemisphere which lies dorsal to the level of the neuroporic recess, we must recognize a differentiation of this region in the reptile's brain into two parts. The dorsal portion is the hippocampal formation and is bounded by the sulcus limitans of Elliot Smith ('03), *s.f-d.* in the figure. The remainder of the area would be regarded as a remnant of the primordium hippocampi of selachians in which differentiation of cortex has not taken place. Within this primordium are found the commissura hippocampi and the upper part of the fornix columns, as is the case in selachians. In the lateral ventricle a groove is seen at the level of the foramen which would be considered as the ventricular boundary of this primordium hippocampi. The body which we are thus hypothetically calling primordium hippocampi is of course the same that has been compared by Meyer ('92) and Unger ('06) with the septum pellucidum of mammals.

As the transverse sections are followed forward this thickening continues but the appearance of distinctness between it and the paraterminal body below gradually disappears. Deferring comment on sections in this intermediate area we may pass to the examination of figure 14. The section is taken behind the olfactory peduncle. The hippocampal formation is clearly marked in the medio-dorsal wall. The cortical layer stops suddenly and a small portion of the medial wall is composed of small cells without regular arrangement. This was called by Meyer part of the septum pellucidum. Below this is a thickening projecting into the lateral ventricle, apparently the same one as was seen near the foramen interventriculare. There can be no doubt of the interpretation of these three portions of the medial wall. The lowest is directly continuous with the tuberculum olfactorium below and receives olfactory tract fibers. It belongs to the area parolfactoria and paraterminal complex. The uppermost is hippocampal cortex. The small body between is undifferentiated primordium hippocampi. The slight sulcus dorsal to it is the sulcus limitans hippocampi of Elliot Smith ('03). Its morphological significance will be discussed later. The deeper sulcus below corresponds to the sulcus limitans hippocampi as that term was used by the present writer ('11 a) for selachians. A cell-free zona limitans extends through the medial wall opposite to it.

The question at once arises, how can the same ridge or thickening in the medial wall be the medial olfactory nucleus at the rostral end of the brain and the primordium hippocampi at the foramen interventriculare, especially since a hippocampal primordium lies dorsal to this ridge at the rostral end of the brain? With the exception of Meyer and of Unger who distinguished between the septum pellucidum and the olfactory nucleus ("vorderes mediales ganglion," nucleus septi), previous authors have considered this whole region as belonging to the paraterminal body. An examination of the entire brain at once shows the error of this assumption. When the lateral ventricle of an adult turtle's brain is opened by removing the lateral wall, the ventricular surface of the medial wall shows the structures seen in figure 19. Rostrally the ventricle is constricted at the level of the olfactory peduncle by a transverse fissure dorsally and by a large longitudinal ridge in the ventro-medial wall. This ridge continues caudad in the lower part of the medial wall and is the medial olfactory nucleus or area parolfactoria seen in figure 14. Above this ridge is a groove in which under a hand lens there are to be distinguished two ventricular sulci corresponding to the two sulci on the medial surface above mentioned (fig. 14). Of these sulci the dorsal one continues caudad in nearly a straight line above the level of the interventricular foramen. The more ventral sulcus, about midway between the olfactory peduncle and the foramen, turns rather abruptly ventrad, forming a rounded caudal boundary to the olfactory ridge, and then bends caudad to reach the interventricular foramen (fig. 19). The body lying between these two grooves is the ridge seen in figure 17 and hypothetically identified above as the primordium hippocampi. It is continuous rostrally with the undoubted primordium hippocampi of figure 14 and is separated from the medial olfactory ridge (area parolfactoria) by a continuous ventricular sulcus. This sulcus has been overlooked by previous workers because they have depended upon the study of sections. After it has been seen in the dissected brain it can be traced in sections (figs. 11 to 17), but owing to its curve it comes to lie at one point so nearly in the plane of transverse sections (fig. 15) as to be very inconspicuous.

The parolfactory ridge above described has been figured by Unger ('06) in sections of the brain of Gecko under the name of nucleus septi, although he describes and figures it as a part of the area parolfactoria. Kappers and Theunissen ('08, fig. 19) call this the nucleus accumbens septi and name the sulcus which bounds it the fovea septo-striatica, considering the nucleus as a part of the striatum. Herrick ('10) cites Kappers and Theunissen but identifies the nucleus in Lacerta (fig. 43) with his nucleus lateralis septi which is in reality part of the primordium hippocampi. That is, he has overlooked the sulcus limitans hippocampi of the above description and has combined the area parolfactoria and the greater part of the primordium hippocampi under the name nucleus lateralis septi.

Upon the medial surface of the hemisphere of a turtle's brain, the outlines of the area under consideration can be made out almost equally well (fig. 20). The rostral portion of the lower half of the wall is occupied by an almost circular area which is continuous with the tuberculum olfactorium in the ventral wall. This clearly corresponds to the medial olfactory nucleus or area parolfactoria of the selachian brain. Dorsal to it are two sulci (cf. figs. 13, 14, 15 and 20). The lower one continues upon the ventral surface as the caudal boundary of the tuberculum olfactorium. The more dorsal sulcus extends from the olfactory sulcus back over the interventricular foramen. These two sulci correspond to the two above described in the ventricular surface of this wall. The more dorsal sulcus is the one called by Elliot Smith the sulcus limitans hippocampi because it forms the ventral boundary of undoubted hippocampal formation. The more ventral sulcus corresponds to the sulcus limitans hippocampi described by the writer in selachians. The part of the medial wall between the two sulci is an undifferentiated portion or vestige of the primordium hippocampi of the selachian brain.

The primordium hippocampi thus outlined contains the commissura hippocampi and together with the hippocampal cortex recognized by previous workers, is the equivalent of the primordium hippocampi of selachians. Compare figure 85.

In Alligator mississippiensis the features described above are repeated so exactly that it is unnecessary to present separate drawings. There are differences in general form, and the area parolfactoria is relatively smaller than in the turtle.

The brains of various mammals, embryonic and adult, have been examined with reference to the gross relations of the primordium hippocampi and the area parolfactoria. In the opossum (Didelphys virginiana) these structures have essentially the same form as in the turtle. The chief difference is that the area parolfactoria is less prominent in the lateral ventricle and the sulcus limitans hippocampi is broader and more shallow. The medial wall as seen from the lateral ventricle is drawn in figure 21, which should be compared with figure 19. The medial surface of the same hemisphere is shown in figure 22. In this it will be seen that the only important difference from the turtle brain is that there are two longitudinal grooves above the foramen interventriculare, one above the fascia dentata, the other below it. Compare Elliot Smith's figure of the brain of Ornithorhynchus ('98, fig. 2). The lower of these sulci separates the fascia dentata from the fimbria and is therefore the fimbriodentate sulcus. The upper one is the fissura hippocampi. The area parolfactoria is prominent but relatively smaller than in the turtle. The primordium hippocampi stretches forward from the dorsal commissure and together with the fascia dentata extends into the medial wall of the olfactory peduncle. Compare figures 25 to 28 and figure 86.

These structures have been studied in a number of mammals. Figures 23, 42, 50, 60, 67, 73 show that in the rat, rabbit, striped gopher, bat, mole, and bear the relations are essentially the same as in the opossum. The same is true in the sheep and foetal dog. The area parolfactoria is smaller in all of these than in the turtle or the opossum, and in some other mammals examined (cat, adult dog, man) this ridge fails to appear in the ventricle. This is due in part to its smaller size (man especially) and in part to the secondary obliteration of the ventricle between the area parolfactoria and the head of the caudate nucleus. In these forms the primordium hippocampi seems to extend down in the medial wall to the floor of the ventricle.

INTERNAL STRUCTURE OF THE MEDIAL WALL

To illustrate the relations in the internal structure of the medial wall of the hemisphere several transverse sections are drawn from the brains of the turtle, opossum, bat, rabbit, rat, mole and bear.

Section through the olfactory peduncle. Figures 12, 25, 41, 48, 56, 65, 75. The matter of interest in this section is the fact already well known from the work of Elliot Smith and others, that the hippocampal formation extends rostrad almost to the formatio olfactoria in most reptiles and lower mammals. The sections selected do not pass through the extreme rostral end of the hippocampal formation but are taken either through the olfactory formation or very close caudal to it. In each section there is seen beneath the ventricle the head of the caudate nucleus, covered ventrally by a thicker or thinner tuberculum olfactorium. The cells of the tuberculum extend up a short distance into the medial wall. Dorsal to this appears a mass of cells which are without regular arrangement and usually are smaller and paler than those in adjacent nuclei. This is the body which has been identified in the above pages as the primordium hippocampi. Above this is a dense mass of deeply staining cells which is continuous caudad with the supra-callosal hippocampus in mammals and with the medio-dorsal cortex in reptiles. This is the rostral end of the hippocampal formation.

Section near genu corporis callosi. Figures 42, 50, 58, 66, 75, 77. Since it is impossible to compare any particular section of the turtle's brain with a section through the genu of the corpus callosum in mammals, the attention of the reader is called to figures 12 to 16, representing sections of the brain of the turtle rostral to the commissures. They are sufficiently described in the explanation of the figures.

In the mole, bat, rat, gopher, and rabbit the precallosal hippocampus approaches the ventral aspect of the genu and continues caudad beneath the corpus callosum to become lost in or fused with the underlying septum pellucidum (undifferentiated primordium hippocampi). This will appear more clearly in the description of sagittal sections below.

As is well known, the hippocampal formation also continues caudad over the corpus callosum. Figures 42, 50, 58 and 66 show the hippocampal formation as it bends around the genu. It is accompanied by precommissural fibers of the fornix system some of which come from farther rostrad while some come up through the septum pellucidum to course around the genu. Below the hippocampal formation and occupying the whole thickness of the wall is the primordium hippocampi. It is small in the rabbit, very large in the mole and in the bat. Below this are the nucleus parolfactorius medialis and nucleus parolfactorius lateralis. Examination of sections farther forward as well as those now under consideration, shows that the nucleus lateralis is in direct continuity around the ventral angle of the ventricle with the nucleus caudatus. The two bodies seem to have the same structure and to form parts of one mass. This is a very conspicuous fact in all the forms examined and has already been noted by Unger and by Kappers. The boundary between the lateral nucleus and the primordium hippocampi is marked by a slight ventricular sulcus, which has been described above from dissections, and a well marked cell-free zona limitans. This zona limitans does not cross the medial wall directly, but at this level appears as a semicircular line of division between the nucleus parolfactorius lateralis and nucleus caudatus internally and the superimposed tuberculum olfactorium externally. Just medial to the zona is seen in most lower mammals either an incomplete and irregularly broken plate of darkly staining cells (Nissl preparations) or a few isolated masses of such cells. These are the islands of Calleja and it is readily seen that they belong with the tuberculum olfactorium in which such islands are numerous. I have never seen these islands rising in the medial wall quite as high as the nucleus parolfactorius lateralis. Medial to these is a more diffuse layer of cells which is continuous ventrally with the superficial layer of the tuberculum external to the islands. This is the nucleus parolfactorius medialis. It is evident that both this and the islands constitute a continuation of the tuberculum olfactorium into the medial wall, as the writer has pointed out in the case of selachians ('11 a). The boundary line between the primordium hippocampi and these two nuclei in the medial wall is somewhat V-shaped as indicated in the figures. The medial nucleus varies greatly in extent but its cells are always imbedded among the fibers of the fasciculus praecommissuralis, forming a more or less dense superficial plate or layer. In the mole and also in the rabbit it comes up from in front almost to the fornix columns; in the bat it rises somewhat higher than the nucleus lateralis, but a large primordium hippocampi intervenes between it and the fornix and the corpus callosum. In the rat the nucleus medialis is small and does not extend as far rostrad as in most forms. In the turtle the nucleus medialis covers the outer surface of the nucleus lateralis with diffusely scattered cells as in the selachian and frog.

The section through the genu in the bear's brain presents a very different appearance from those described above, owing to the great development of the frontal lobe which occupies the space in this section between the olfactory bulb and the genu. In figure 73, however, is drawn a section between the genu and lamina terminalis in which the relations of the primordium hippocampi, nucleus parolfactorius lateralis and nucleus parolfactorius medialis are seen to be as described above. In this section the indusium appears above the corpus callosum and the corresponding cord of gray beneath it.

Section through the neuroporic recess. Figures 17, 29, 44, 51, 62, 69, 70, 72. This section cuts at the same time the anterior commissure, the fornix columns and some part of the anterior pallial commissure complex, and passes just rostral to the foramen interventriculare. Some attention must be given separately to each of the forms studied.

In the turtle (figs. 9 and 17) the neuroporic recess is nearly obliterated in the adult, apparently through the anterior and pallial commissures approaching one another in later embryonic stages. The dorsal commissure has the form of a loop whose two limbs rise dorsally at either side to reach the hippocampus (figs. 9 and 16). All recent authors agree that the compact layers of cells occupying the medio-dorsal wall of the hemisphere represents some part of the hippocampal formation of mammals. Meyer ('92), Elliot Smith ('96) and Levi ('04) rightly hold that the lower portion of this cortex is the forerunner of the fascia dentata. The ventral boundary of the hippocampal formation is sharply marked both by the sudden change to a body containing cells irregularly scattered through the thickness of the wall and by a well-defined sulcus. The mass of scattered cells is the primordium hippocampi above described. Its dorsal portion in this section is filled by fibers of the fimbria system. This is an old system of fibers connecting olfactory centers in front with the whole length of the hippocampus. It is well developed in the embryo before the hippocampal commissure is formed. The constitution of this precommissural representative of the fimbria is discussed below. The sulcus above mentioned, since it lies between the fimbria and the developing fascia dentata, must be regarded as the homologue of the fimbrio-dentate sulcus of mammals. This sulcus has been variously treated by previous authors. Elliot Smith ('03) calls it the sulcus limitans in Sphenodon, Edinger ('96) and Unger ('06) call it the fissura arcuata, Kappers and Theunissen ('08) call it fissura septo-corticalis, de Lange ('11) uses the same name but calls it also fissura arcuata. Herrick ('10) discusses the matter, and retains the term fissura arcuata. The sulcus in question can not be the fissura arcuata, since that is situated within the hippocampal formation, dorsal to the fascia dentata. The fissura arcuata is not present in the reptiles studied by the writer. On the other hand, the fimbrio-dentate sulcus is a constant feature in the brains of reptiles and mammals. It is the sulcus shown by Elliot Smith ('98, pl. XI, '10, fig. 5) in the brain of Ornithorhynchus beneath the fascia dentata and seen in the same position in the brain of Didelphys and the bat (figs. 22, 30, 43, 44). Elliot Smith ('03, p. 469) gives this sulcus the name sulcus limitans hippocampi and points out the error of writers on the reptilian brain who regard this as the fissura arcuata. In higher mammals the growth of the corpus callosum greatly disturbs this sulcus in the rostral half of the hemisphere but the fimbrio-dentate sulcus is a well known landmark in that region of all mammalian brains where the primary relations of hippocampal formation and fimbria are retained. It persists throughout the length of the corpus callosum in the bear (fig. 76) and the bat (figs. 43, 44).

The sections of the turtle brain show that the body which Meyer called septum pellucidum and which I have here called primordium hippocampi is marked off from the area parolfactoria by a ventricular sulcus and a cell-free zone, is traversed by the olfacto-cortical fibers and by the hippocampal commissure. This body lies above the neuroporic recess and in all these respects it agrees with the primordium hippocampi of selachians. The only difference is that a part of the hippocampal primordium of selachians has developed into hippocampal cortex in reptiles.

In Didelphys (fig. 29), as in the marsupials described by Elliot Smith, the dorsal or anterior pallial commissure lies in the same plane with the large anterior commissure and above the neuroporic recess. The mass of gray matter in which it is partly imbedded projects into the ventricle and is separated from the parolfactory nuclei rostrad by the ventricular sulcus limitans in the manner described above. It is the residue of the primordium hippocampi of selachians and is continuous with the fascia dentata and hippocampus above. The fibers of the anterior pallial commissure bend dorsad through the primordium to emerge on the ventricular surface of the hippocampus as the alveus. Above the commissure is the recessus superior whose membranous walls are attached to the dorsal surface of the commissure. This is nothing more or less than a rostral pocket of the third ventricle covered by tela chorioidea, formed by the commissure pushing into the ventricle as a transverse ridge. Just lateral to the attachment of the tela is the fimbrio-dentate sulcus. Above the fascia dentata the deep fissura hippocampi presents the typical mammalian relations. The constitution of the anterior pallial commissure is discussed in a later section.

In the bat (fig. 44) the section cuts the two commissures very much as in the opossum. Above the anterior pallial commissure is a well developed hippocampus and fascia dentata. The hippocampal fissure and the fimbrio-dentate sulcus are similar to those of Didelphys. The lateral ventricles are reduced to very narrow slits. The medial wall of the ventricle consists of a primordium hippocampi in which the pallial commissure is imbedded as in all other forms.

In the mole (fig. 51) the section cuts the large anterior commissure, the fornix columns and the corpus callosum. Above the corpus callosum is the indusium with the stria Lancisii, and beneath it the primordium hippocampi which is relatively larger than in any other mammalian brain studied. Between the fornix columns it contains large cells comparable with the large pyramids of the hippocampus. The relations of the neuroporic recess are commented upon in connection with the sagittal section, which shows them better.

In the rabbit (figs. 69, 70) the general relations are the same as in the mole. A very small indusium appears above the corpus callosum. The septum pellucidum of authors consists of a diffuse gray mass which is directly continuous forward with the primordium hippocampi. Just rostral to the neuroporic recess each lateral half of this primordium is almost semicircular in outline (fig. 69). The fornix columns come up through it, a large part of their fibers turning laterad as the body of the fornix to become continuous with the fimbria, another large part ascending to take a place immediately beneath the corpus callosum near the median line in which position the fibers continue caudad to be distributed to the hippocampus. This is the fornix superior of Kölliker and Elliot Smith. The fornix body divides at the level of the neuroporic recess into dorsal and ventral portions. The dorsal portion forms a thin covering for that part of the hippocampus which extends farthest rostrad beneath the corpus callosum (hippocampal flexure). This appears in sections immediately behind the foramen and thus overlaps dorsally the primordium hippocampi in which the commissure is imbedded. The ventral portion becomes the fimbria proper.

In the bear (fig. 72) the neuroporic recess projects rostrad between the fornix columns somewhat beyond the rostral border of the anterior commissure. Above the recess the space between the fornix columns is occupied by the hippocampal commissure. Above this are the bundles of the fornix superior and among them a little gray matter which is the continuation of the subcallosal hippocampus traced back from the level of the genu. At about this level this small mass of gray becomes fused with the primordium hippocampi (septum). The latter body is here largely filled with the fibers of the fornix system. Its ventricular portion is relatively free from medullated fibers. On the dorsal surface of the corpus callosum is the indusium with the striae Lancisii which will be more fully described in a later section.

The rat possesses the largest hippocampal formation rostral to the foramen interventriculare that the writer has seen in any mammal. At the olfactory peduncle (fig. 56) a very broad area of deeply staining cortex is seen on the medial surface almost in contact with the olfactory formation. This is reduced to a narrow band at the level of the rostral border of the caudate nucleus (fig. 57). Here is a great development of the deep layer of the tuberculum and between this and the cortex mentioned is a narrow area of small pale cells which represent the primordium hippocampi. At the rostral border of the corpus callosum (fig. 58) the special enlargement of the deep layer of the tuberculum has disappeared and the islands of Calleja invade the lower part of the medial wall. The primordium hippocampi is much increased in size while the band of cortex previously described is still small. Curving about the genu of the corpus callosum is seen well developed cortex accompanied by the stria medialis Lancisii. Nine sections farther caudad (fig. 59) the two bands of hippocampal cortex have united into a broad band beneath the corpus callosum, and above the latter is a rather large indusium. The primordium hippocampi is broad and is bounded below by very large islands of small cells which take a very deep stain. At this level the primordium hippocampi includes a large mass of gray between the sub-callosal hippocampal cortex and the ventricle. It is separated from the nucleus parolfactorius lateralis by an oblique zona limitans. The nucleus lateralis appears to be merely the medial part of the head of the caudate nucleus which does not extend into the medial wall beyond the lower angle of the ventricle. This relation is more striking in the rat than in any other mammal studied. Fifteen sections caudal to the last figure the very broad sub-callosal hippocampal cortex has become reduced to a small band (fig. 60) but the primordium is still large and bears the same relation to the nuclei parolfactorii medialis and lateralis and to the islands of Calleja as in other forms described above. Caudal to this (fig. 61) the cortical band merges into the primordium, which maintains the usual relations. At the level of the neuroporic recess (fig. 62) all the relations are closely similar to those in the rabbit. Caudal to the foramen the primordium extends a short distance among the fimbria fibers and then is continued as a band of cells flattened between the hippocampal commissure and corpus callosum, accompanying the fornix superior as in the rabbit and bear. These cells eventually are lost to view among the cells of the deep or ventricular layer of the hippocampus.

Relation of primordium hippocampi to true hippocampus caudal to the foramen interventriculare. The fact has been mentioned above that in the rabbit the hippocampus extends rostrad beneath the corpus callosum almost to the level of the foramen and overlaps the primordium hippocampi. The commissure and fornix are imbedded in a continuous mass of gray which has reached the cortical stage of differentiation behind the commissure, while in front it remains an undifferentiated primordium. The same is true of the mole. Here the hippocampus proper extends rostrad only a short distance beneath the splenium (fig. 88) but the primordium continues caudad over the foramen among the fimbria fibers and as a mass of pure gray matter on the ventricular surface of the fimbria until it overlaps the rostral end of the hippocampus. Here the primordium and the cortex meet as a common bed for the fimbria and the caudal portion of the hippocampal commissure which lies beneath the splenium. The condition in the rat also indicates the essential continuity of the primordium hippocampi and the hippocampus proper. This subject is more fully discussed in the following section.

In the turtle (fig. 18) the primordium hippocampi behind the foramen is small and is occupied by the fimbria. The fimbrio-dentate sulcus continues back in the medial wall of the hemisphere as far as the fimbria can be recognized, some distance beyond the end of the choroid fissure (fig. 20).

EXAMINATION OF MEDIAL SURFACE OF HEMISPHERES AND OF SAGITTAL SECTIONS

The medial surface of the opossum's hemisphere is drawn in figure 22. The reader will notice the deep hippocampal fissure above the fascia dentata and that it is suddenly obliterated rostrad by the deep in-folding of the dorsal wall at the sulcus olfactorius. The fascia dentata is narrow over the anterior pallial commissure and grows wider rostrad. Beneath it is the broad bundle of precommissural fibers of the fornix system. The anterior pallial commissure stands in the path of these fibers. The fimbrio-dentate sulcus begins between the dorsal end of these fibers and the fascia dentata and extends caudad over the commissure. The locus of the neuroporic recess is clearly seen in the so-called recessus inferior. Upon this surface no sulcus is visible between the primordium hippocampi and the area parolfactoria. It has probably been obliterated by the great bundles of precommissural fibers running dorso-ventrally across it. The course of the boundary line, as indicated by the internal structure above described, is shown in figure 86.

In figures 35 and 34 are drawn a nearly median sagittal section through the region surrounding the commissures and a section of the anterior pallial commissure and hippocampal formation lateral to the median plane. In the most medial section it is seen that the pallial commissure lies in a lamina supraneuroporica containing gray matter and that the tela is attached to the dorso-rostral border of this, forming the recessus superior. The projection of the chief volume of the lamina supraneuroporica into the ventricle is a characteristic difference between marsupials and higher mammals. In the bat (fig. 45) an intermediate condition is found. The pallial commissure has already the crescent shape which is characteristic of those forms in which the corpus callosum has begun to separate from the hippocampal commissure. In the caudal thick part of the commissure are found a number of small bundles of non-medullated or lightly medullated fibers and the rostral part of the crescent seems to contain many lightly medullated fibers. This rostral horn of the crescent of course forms the precommissural portion of the alveus. Whether the lightly medullated fibers have different functions from the heavily medullated can not be decided at present. In the section lateral to the median plane it is seen that the primordium hippocampi is directly continuous with the fascia dentata through the commissure bundles (figs. 33, 34). The cells of the fascia dentata with which the primordium comes into relation are those in the concavity of the fascia dentata which Kölliker ('96, p. 737, fig. 777) and Edinger ('04, p. 333 and fig. 232) call the end-portion of the layer of pyramidal cells of the hippocampus. In transverse sections this continuity of the primordium with the hippocampal formation is not conspicuous because the alveus seems everywhere to form a complete partition of fibers between the two gray masses. In sagittal sections it is seen at once that the alveus is in bundles between which columns of cells connect the two masses; or, rather, that the two form one continuous mass which is traversed by the alveus bundles.

When transverse sections stained by a cell stain are studied more carefully instructive facts are brought out. First, as is well known from the work of Elliot Smith and Levi, the fascia dentata in its rostral part is directly continuous with the ventral border of the hippocampal cortex, as seen in a transverse section (fig. 28). In both hippocampus and fascia dentata is seen a sparse layer of polymorphous cells next to the ventricle, in part mingled with alveus fibers. In the rostral part of the fascia dentata this ventricular layer consists of a much larger number of cells and these are broadly continuous with the mass of the primordium hippocampi (figs. 28, 32). Further caudally, where the fascia dentata becomes displaced so as to embrace the border of the hippocampus in its concavity, the cells which fill this concavity are seen in favorable sections in direct continuity with the primordium through the alveus (figs. 29, 30, 31). From these facts it appears that the polymorphous layer contains the primitive cells of the hippocampus and is comparable to the primordium hippocampi. This is especially clear in the relations of the primordium to the hippocampal cortex in the pre-callosal region in the rat (figs. 58, 59).

The brain of the bat is rather small for dissection and the writer has not had a sufficient number of specimens to warrant using them in this way. Therefore the median plane of this brain is reconstructed from sagittal sections and a parasagittal section is drawn to illustrate the arrangement of centers in the medial wall (figs. 45, 46). These figures show clearly that while the nucleus parolfactorius medialis extends high up toward the pallial commissures it does not reach them but these commissures are imbedded in a considerable mass of gray matter which lies dorsal to the level of the neuroporic recess. This mass is the primordium hippocampi and is separated from the area parolfactoria below by a well marked cell-free zona limitans hippocampi. The primordium consists of two parts, a dense collection of small cells forming a bed for the hippocampal commissure (fig. 46), and a much less dense area of larger cells beneath the corpus callosum. Rostrad the zona limitans rises almost to meet the genu of the corpus callosum, but in sections to one side of the median plane (fig. 47) the supra-callosal hippocampus (indusium) surrounds the rostral border of the commissure and becomes continuous with this sub-callosal portion of the primordium hippocampi. Rostral to the genu the hippocampal formation extends to the olfactory peduncle. Close to and in the median plane the large celled portion of the primordium is reduced to small size but is distinct from the nucleus parolfactorius medialis. Caudally the commissure is imbedded between the primordium and the hippocampus proper and dorsally the cells of the indusium are occasionally found intermingled with the bundles of the corpus callosum (fig. 47) so as to establish a continuity between the indusium and the primordium. In front of the commissure there is a broad continuity of these bodies as already described. It thus appears that the corpus callosum and hippocampal commissure are imbedded in a continuous mass of gray matter which occupies the thickened lamina supraneuroporica and extends into either hemisphere as the hippocampal formation. At the point *m* where the tela chorioidea is attached to the caudal surface of the hippocampal commissure is a prominent module of cells to which Elliot Smith ('97 c) has called attention in Nyctophilus. This is the primary upper border of the lamina supraneuroporica and the cells belong to the indusium verum as defined by Professor Smith ('97 e). It might be called nodulus marginalis.

In the rat there is a large corpus callosum with well developed genu and splenium. The hippocampal commissure is a plate of fibers broad dorso-ventrally and standing nearly vertically beneath the body of the corpus callosum, a little nearer to the genu. At its dorsal border the plate of fibers bends caudad beneath the corpus callosum and becomes continuous with the thin edge of the splenium (fig. 89). This form of the commissures is apparently due to the dorsal end of the large hippocampus which fills the angle between the hippocampal commissure and the splenium, the hippocampal flexure of Elliot Smith. The pillars of the fornix are large and rise in front of the hippocampal commissure relatively free from mingling with it (fig. 64). Just beneath the corpus callosum they turn latero-caudad in the fimbria. Between the fornix columns and the hippocampal commissure, and to some extent mingled with the latter, is the small-celled nucleus already described in the bat. Beneath the rostral part of the corpus callosum the septum pellucidum is filled with large cells as in the bat. Here in the rat these cells become more compactly arranged near the median line and are directly continuous with the indusium around the genu as described above from transverse sections. Rostral from the genu the indusium is continued by a cortical band which reaches to the olfactory bulb. Beneath this is a band of lightly staining cells, loosely and irregularly arranged, which represents the primordium hippocampi. It is directly continuous with the less compact part of the septum pellucidum above described. Finally, this part of the septum pellucidum in its caudal and lateral part is connected with the hippocampus proper by columns of cells between the bundles of the hippocampal commissure as in the opossum. See the description of figures 63 and 64. There is therefore a continuous formation which begins at the olfactory peduncle, divides at the genu into indusium and septum pellucidum and unites again in the hippocampus behind. In other words, the hippocampal and callosal commissures are imbedded in one formation which in part is developed into hippocampus and in part remains in the primitive condition. The septum pellucidum includes the larger part of this undeveloped primordium hippocampi.

The median plane of the brain of the mole is reconstructed in figure 55. There is an enormous massa intermedia and the third ventricle between it and the anterior commissure is reduced to a very narrow slit. The hippocampal commissure is nearly horizontal in position and the septum pellucidum very narrow dorsoventrally. It is largely occupied by fibers of the fornix superior. The feature of really striking importance about this section is the existence of a band of non-nervous tissue leading from the neuroporic recess out to the surface below the genu of the corpus callosum. In Weigert sections this has almost the appearance of a canal and is occupied by blood vessels. In the sections at either side of the median plane the fibers of the precommissural bundle, which are interrupted in this figure, pass on into the fimbria. When this is compared with the condition in the mammalian embryo it is evident that this is a vestige of the great sagittal fissure which has been closed up by the encroachment of the paraterminal body below and the pallial commissures above. If this figure be compared with the sagittal sections of the bat's brain, it will be seen that a quite similar line of demarcation between the paraterminal body and the septum occurs in the bat and that the cell-free space is occupied by blood vessels.

Werkman's photographs of sagittal sections of this region in bat embryos are extremely instructive in this connection (Werkman '13, figs. 13, 17 and 18). These show the relations between the paraterminal body and the septum in three stages. Essentially the same condition is seen in the embryos of the rabbit, cat and other mammals. If now we turn to the selachian and compare Werkman's figure 18 and figures 45 and 55 of the present paper with the median section of Scyllium (fig. 84) we find an almost complete correspondence of part for part. The external neuroporic recess in the selachians is a deep canal containing blood vessels. In some selachians the canal is obliterated and the vessels are imbedded in a solid mass of tissue. An external pit is retained in the adult bat and mole and in all embryos. In the bat and mole a cell-free and fiber-free band occupied by blood vessels marks the position of the external neuroporic recess. The same is true but less prominent in some other mammals. In the human embryo of 31 mm. the external neuroporic recess is penetrated by a special group of blood vessels (fig. 8). The mole and the bat show in a striking manner that even when there is a large corpus callosum, as in the mole, the essential relations of the area parolfactoria and the hippocampal formation with its pallial commissures do not differ at all in selachians and mammals.

One other point in the brain of the mole may be mentioned here. When the region of the splenium is studied in transverse sections it is seen that the enlargement of the indusium behind and beneath the splenium (fasciola cinerea) consists of much larger cells than those in the adjacent hippocampus proper (fig. 54). These cells form a plate which is continuous at its lateral edge with the layer of pyramids of the hippocampus. The fascia dentata takes no part whatever in the formation of the indusium. As the hippocampus is followed forward beneath the corpus callosum the cells of the fasciola cinerea gradually blend to some extent with those of the hippocampus. This plate of cells is, however, readily followed forward beneath the hippocampal commissure until the fascia dentata disappears from the section and this plate of cells merges with the septum pellucidum as above described (fig. 53).

CONSTITUTION OF DORSAL COMMISSURE

Symington ('93) and Elliot Smith ('97 d, '02, '03 b) have presented evidence that the marsupials do not possess a true corpus callosum. The corpus callosum of mammals is defined as consisting of fibers coming from undoubted neopallial areas, invading the alveus of the hippocampus and crossing to the opposite hemisphere through the dorsal commissure. Elliot Smith ('03) believes that while such a commissure does not exist in marsupials, the function of a corpus callosum is performed by fibers which cross in the anterior commissure, reaching it by way of the external capsule. The writer has at hand at the time of writing only Smith's reply to Zuckerhandl (Smith '03 b). As this clearly defines the questions involved it will be of interest to note some evidence bearing on the subject derived from the brain of the opossum. In Weigert sections corresponding to the one from the brain of Perameles beautifully figured by Elliot Smith the writer can not doubt that fibers enter the alveus from the medio-dorsal cortex far beyond the transitional area between hippocampus and general cortex (fig. 37). Sagittal sections show this more clearly (fig. 39). Since Weigert sections are not conclusive on a point like this, an attempt was made to test the presence of callosal fibers in the dorsal commissure by experiment. The attempt in two operations to cut the dorsal commissure without injury to anything else failed, but in other experiments light was thrown on this question. In one animal an area of dorsal cortex (shown in fig. 40, *a*) was scraped out. The animal when killed showed no infection and there is in the sections no evidence of any injury being done to the hippocampus or alveus. Preparations were made by the Marchi method and showed the following results: internal capsule deeply degenerated; external capsule affected; anterior commissure not affected at all; alveus and dorsal commissure contain many degenerated fibers, traced from the lesion (fig. 40).

In another animal a much larger area of dorsal cortex was destroyed (fig. 40, *a*). Although the internal capsule was badly degenerated and the external capsule likewise, no degeneration is seen in the anterior commissure. The dorsal commissure shows degenerated fibers, but in this case there was possibility of direct injury to the alveus.

In a third animal in which a large lesion was made in the thalamus and midbrain, there was incidental injury to the dorsomedial angles of the hemispheres. On the left side this invaded the hippocampus secondarily, largely destroying its dorsal portion; on the right it affected only the dorsal cortex.. The hippocampal commissure was of course deeply affected and both sides show degeneration in the internal capsule. The anterior commissure shows no degeneration. The character of the degeneration in the dorsal commissure differs as the lesion affects the hippocampus or the general cortex. After hippocampal lesion the degeneration is more profuse and the blackened droplets are much coarser. This is perhaps to be explained on the supposition that fibers arising in the general cortex are more lightly medullated. Attention has been called to the fact that the dorsal commissure contains bundles of lightly medullated and nonmedullated fibers. A similar explanation can scarcely be given for the apparent absence of fibers from the dorsal cortex in the anterior commissure. One receives the impression from Weigert sections that the anterior commissure consists almost wholly of medullated fibers.

It thus appears that in the opossum we have positive evidence in Weigert and Marchi preparations for the presence of corpus callosum fibers in the dorsal commissure and negative evidence from Marchi preparations regarding fibers from the dorsal cortex crossing in the anterior commissure. Further study of the distribution of the anterior commissure is under way.

The facts above stated warrant a reinvestigation of the Australian marsupials. The presence of true corpus callosum fibers in marsupials and also in reptiles is to be expected in view of the fact that in fishes, amphibians and reptiles sensory radiations ascend from the thalamus to the telencephalon and of the further fact that in selachians the telencephalic center for these thalamic radiations is connected with its fellow by a commissure which bears the morphological relations characteristic of the mammalian corpus callosum. It is worthy of note that Unger ('06) describes in the gecko a large part of the dorsal commissure going to the cortex far lateral to the hippocampus. These fibers can scarcely be other than corpus callosum fibers. Pedro Ramon ('94) has also figured fibers in Lacerta, which must be callosal fibers if the figure is accurate. See Elliot Smith's discussion of this ('03, p. 482). See also Cajal ('04, p. 1103), who states that these are callosal fibers. In the turtles studied the lack of medullation in the dorsal commissure has made it impossible thus far to secure positive evidence as to the presence of callosal fibers.

DEVELOPMENT OF PALLIAL COMMISSURES

The development of the pallial commissures has been the subject of extensive phylogenetic and ontogenetic studies by numerour authors. For the general course of evolution of the two commissures we are indebted chiefly to the comparative researches of Elliot Smith. The studies of embryonic development by Schmidt ('62), Mihalkovics ('77), Blumenau ('91), Marchand ('91), Martin ('93), His ('89, '04), Hochstetter ('98), Zuckerkandl ('01, '09), Grönberg ('01), and Goldstein ('03), have given conflicting results on certain points. Elliot Smith maintained that the fibers of the corpus callosum in mammals entered the commissure bed which already contained the hippocampal commissure, that the callosal fibers became segregated in the rostral limb of a crescent-shaped commissure, that the growth of the general cortex was followed by an increase of the callosal fibers, that these fibers caused an expansion and stretching of the commissure bed and that the entire hippocampal-callosal commissure system remains in higher mammals surrounded by the vestiges of the primary commissure bed. Mihalkovics, His and Zuckerkandl have held that the corpus callosum forms in a secondary area of fusion of the medial walls of the hemispheres. This is opposed by Goldstein who believes that in man the primary commissure bed is expanded by the callosal fibers growing into it. There has just come to hand as I write the study of Werkman ('13) who finds this to be true in Vesperugo, Erinaceus, and Talpa. The writer has studied carefully the development of the corpus callosum in pig embryos and must agree with Goldstein and Werkman. The intermediate view of Marchand, that there is a very early fusion of a small area of the hemisphere walls which is thereafter stretched by the growth of the commissures may be explained by two factors. First is the thickening of the lamina terminalis (and of the lamina supraneuroporica as defined in the present paper) by the proliferation of neuroglia as described in detail by Werkman. This results in a thickened "Glia-shicht" through which the fibers may run. Second, there is a migration of cells (neuroblasts) into the lamina supraneuroporica from the hippocampal primordia at either side just as there is a secondary thickening of the lamina terminalis by migration of cells from the paraterminal body to form a bed for the anterior commissure. In figures 7 and 24 is seen a ridge extending upward and forward from the lamina supraneuroporica in the hemisphere wall. This ridge indicates the line along which pallial (hippocampal) commissure fibers enter the lamina and cells migrate into this lamina to form the commissure bed described above in several mammals. This thickening of the roof plate at first by glia and afterward by neuroblasts produces the massive lamina supraneuroporica which is characteristic of vertebrates and may explain Marchand's conception of an early area of fusion and Grönberg's concrescentia primitiva. Space can not be taken for a full account of the development of the commissure. The reader is referred to the description of figures 78 to 81.

That there is no fusion of the medial walls and that the commissure bed is merely expanded by intussusception of callosal fibers is evidenced by the appearance of the commissures in the lamina supraneuroporica (upper border of lamina terminalis of authors) (Marchand, figs. 7, 9; Werkman, figs. 5, 17, 24); the fact that the corpus callosum in all stages of growth has a smooth and regular border upon which the falx and anterior cerebral artery lie as if they had been pushed before the developing commissure (Goldstein, p. 46 and fig. 15); and the fact that the commissures are always completely surrounded by a thicker or thinner layer of nerve cells (Blumenau); which I can confirm from my own studies. The last point is of great importance. It has been shown in a previous section that the corpus callosum and hippocampal commissure in the opossum, bat, mole, rat and rabbit are everywhere surrounded by the hippocampal formation and the primordium hippocampi. The cells of the indusium verum of Elliot Smith and the cells among which the commissure fibers are imbedded must be regarded as the remnant of the cells which enter the lamina supraneuroporica from the adjacent hippocampal primordia in early stages of the embryo. Thus the anterior pallial commissures in mammals are imbedded in the primordium hippocampi just as they are in selachians.

STRIAE LANCISII AND INDUSIUM

To the very concise and illuminating survey of the history of these structures given by Elliot Smith ('97 e) I can add a few interesting comments on the basis of the forms studied. Great differences appear in these structures in various mammals. In most of the rodents the indusium is small and the morphological arrangements seen in the better developed hippocampus are lost. In the woodchuck (Arctomys) there is a fairly thick band of cells continuous across the median line and covered by a layer of fibers. This probably indicates a fusion of the indusium and striae of the two sides. The striped gopher (Spermophilus tridecemlineatus) agrees with the woodchuck. A very different condition appears in the opossum and the bear. I have seen no mention made of a stria Lancisii in the marsupials and none was to be expected on the hypothesis that this bundle represents fibers which run within the hippocampal formation when that is well developed. Elliot Smith states that in Eutheria "the vestigeal fascia dentata lies in the region of the stria medialis." I was surprised, therefore, to find in transverse sections of the opossum's brain well defined striae mediales on the dorsal surface of the pallial commissure. As shown in figures 29, and 30, these are good-sized dense bundles lying at either side of the middle line and separated from the fascia dentata by the fimbrio-dentate sulcus already described. Traced caudad these bundles curve down on the caudal surface of the commissure and turn laterad.

They are then lost in the commissure itself and presumably go to the hippocampus. Rostrally these fibers bend down over the rostral border of the commissure and are made up from the fibers of the fasciculus praecommissuralis (figs. 28, 35, 36). From the stria medialis in its longitudinal course small bundles are given off to the deep layer of the dentate fascia and the hippocampus (fig. 36). These are in all probability fibers from the tuberculum and other olfactory centers to the hippocampus. The fact that the stria is separated from the fascia dentata by the fimbriodentate sulcus at once suggests comparison with the fimbria in reptiles. The stria medialis undoubtedly corresponds to a part of the reptilian fimbria and it is important to notice that it runs in the primordium hippocampi (septum) in the opossum as in the reptile. The stria lateralis is represented in the opossum by the longitudinal fibers in the stratum zonale of the hippocampus as Elliot Smith has stated. In addition to longitudinal association fibers of the hippocampus, however, these bundles include many fibers which come up from the tuberculum through the rostral part of the primordium hippocampi. These are fibers of the same category as those which make up the stria medialis; both are olfacto-cortical fibers. These run near the lateral border of the hippocampus, while the stria medialis runs along its medial border. Both the olfacto-cortical and the association fibers are seen in the mole (fig. 48) but most of them end in the precallosal hippocampus and primordium because the supracallosal hippocampus is reduced to an indusium in the mole. In the rabbit the stria medialis curves around the genu and is lost among the fibers of the precommissural fasciculus rather far back beneath the corpus callosum.

In the bear the indusium is rather large and maintains the relations seen in the opossum. As seen in figures 76 and 77, the stria medialis is separated from the hippocampus by a fimbrio-dentate sulcus, the fascia dentata and hippocampus can be distinguished and in the rudimentary hippocampus are seen both the vestigeal alveus and the superficial stria lateralis. These structures curve about the genu and are well preserved beneath the callosum until the hippocampus expands into a broader band reaching to the olfactory peduncle (figs. 77, 75).

In the bat also the stria medialis is made up of precommissural fibers and runs independently of the fascia dentata, separated from it by the fimbrio-dentate sulcus (fig. 43). Caudally the fibers pass into the lateral part of the nodule of cells at *m*, and are lost. Probably they turn laterad here in the hippocampal commissure.

The above facts seem to show conclusively that the indusium and stria lateralis Lancisii are strictly vestiges of the hippocampus and fascia dentata as those exist in the marsupials. The stria medialis, however, is a separate structure present in the marupial and comparable with the fimbria bundle in the reptiles. Professor Smith ('97, p. 85) speaks of the stria medialis as "the fimbria of the dorsal hippocampus." It together with the pallial commissure belongs in the septum or undeveloped hippocampal primordium. The stria medialis is strictly a part of the fornix longus as defined by Elliot Smith ('97, p. 88) but differs from all the other fibers of the fornix system in that it does not pierce any part of the pallial commissure complex in order to reach the hippocampus. This is recognized by Elliot Smith in the passage referred to, which reads:

> From this account of the arrangement of fibers in the ox-brain, it is evident that *all the longitudinal uncrossed fibers of the fornix break through some part of the great dorsal commissure* (psalterium, splenium, or corpus callosum, as that term is generally understood) *in order to reach the septum.* These fibers constitute the true *fornix longus.* The *fornix superior* consists of those fibers of the fornix longus which do not pass through the main mass of the psalterium, but break through a commissure of non-hippocampal or a mixture of the latter and hippocampal fibres (i.e., corpus callosum and its splenium).
>
> A few fibers of the fornix do not pass through any commissure. In the marsupial (fig. 4a) these fibers spring directly from the most anterior part of the hippocampus, and pass downwards to their destinations in front of the commissures. The corresponding fibers in the higher mammal (fig. 2a) become pushed forward by the extending genu corporis callosi, but their essential disposition is unaltered, i.e., they spring from the anterior extremity of the stria mesialis Lancisii.

This statement requires modification in that the stria medialis is connected with the caudal as well as the rostral part of the hippocampus. The opossum and the bat show this conclusively. It is probably for the very reason that the stria medialis is related to the caudal part of the hippocampus that it is preserved at all in higher mammals. Certainly it curves about the splenium to enter the fully developed hippocampus in all the forms that I have studied. It is undoubtedly made up chiefly of the very primitive system of olfacto-cortical fibers which are already seen in (cyclostomes and) selachians and which constitute most of the fimbria bundle in the rostral part of the reptilian hemisphere. The fibers of this system which enter the rostral part of the hippocampus nearly all disappear in mammals possessed of a large corpus callosum so that the stria lateralis Lancisii consists chiefly of hippocampal association fibers and is sometimes difficult to recognize in these higher forms.

Elliot Smith ('97, p. 55) suggested that the fasciculus marginalis described by him in Ornithorhynchus is represented in higher mammals by the stria medialis. The fasciculus marginalis in the opossum goes in part into the stria medialis and in part into the "association bundle" in the stratum zonale of the hippocampus. Since the latter is practically lost in higher mammals it would be true to say that the fasciculus marginalis is represented by the stria medialis. On the other hand, the stria medialis is not wholly made up of the fasciculus marginalis, since it receives fibers from the other parts of the precommissural system.

Two other points are worthy of notice in this connection. First, the relations in the bear show clearly that the sulcus corporis callosi in higher mammals is the fissura hippocampi, strictly comparable to that of the marsupial or the bat. And this suggests that when the perforating fibers of the fornix superior appear to go to the cingulum they are in reality only taking a position along the lateral border of the reduced hippocampus. The hippocampus is sometimes enlarged and the hippocampal fissure better developed in front of the corpus callosum or beneath the genu, as in the bear and the rat (figs. 58, 77). Second, the recognition that the stria medialis is independent of the hippocampus proper affords us a definite boundary line in most mammals between the hippocampal formation and the residue of the hippocampal primordium (i.e., septum, pallial commissures and fimbria system). This boundary line is marked by the fimbriodentate sulcus. This adds needed emphasis to the fact that the corpus callosum does not itself mark this boundary.

GENERAL RELATIONS OF FIBER TRACTS AND COMMISSURES IN THE SEPTUM OF VERTEBRATES

These relations are readily understood in view of the considerations in the last section and of the conditions in selachians. In these fishes (fig. 91) the pallial commissures are imbedded in the hippocampal primordium, the corpus callosum dorsal to the hippocampal commissure. Interwoven with both commissures are longitudinal fibers of the tractus olfacto-corticalis, while the columns of the fornix emerge from among the fibers of the hippocampal commissure to descend at either side of the neuroporic recess as in reptiles and mammals. When in these higher forms a part of the hippocampal primordium becomes hippocampus the commissures remain imbedded in the undifferentiated primordium and the longitudinal fibers reach the hippocampus through the residual primordium, retaining essentially the same relations among themselves. As the hemisphere elongates, the olfacto-cortical system takes on the form of a more compact bundle, the precommissural fimbria. This is joined by the fibers from the nuclei of the lateral olfactory tract running by way of the anterior perforated space and helping to form the fasciculus precommissuralis. This bundle contains according to Elliot Smith ('97) and Cajal ('04) many fibers which arise and others which end in the septum. The efferent fibers go in part at least to the hypothalamus. The fibers which end in the septum belong chiefly to the olfacto-cortical system. A study of the precommissural system in the opossum and the rabbit shows that the fibers come chiefly from the olfactory tubercle, anterior perforated space, nucleus of the lateral olfactory tract and perhaps the whole pyriform lobe. It is the great system of fibers by which impulses are carried from the area olfactoria to the hippocampal formation. Since many of these fibers end in the septum, the fiber relations of the septum are not unlike those of the hippocampus itself.

The relations of the commissures to the fimbria, fornix superior and the stria Lancisii are surprisingly primitive. In marsupials and lower mammals, as in selachians, the two commissures are interwoven with longitudinal fibers of the fimbria system (olfacto-cortical) on their way to the hippocampus. When the corpus callosum increased in size, separated from the hippocampal commissure and arched dorsally to form the splenium, it carried up on its dorsal surface such fibers as ran over it. These are the stria medialis Lancisii. The fibers which were interwoven with the callosal fibers have retained that relation and are the fornix superior and perforating fibers of Kölliker; the fibers (olfacto-cortical and fornix columns) which were primitively interwoven or intermingled with the hippocampal commissure have retained that relation and form the mammalian fimbria.

This interpretation is illustrated in figures 92 and 93 showing the relations of the fornix system to the commissures in the bat, which is intermediate between the marsupials and higher mammals, and in the rat, which presents a commissure system essentially like that of man. Figures 35 and 38, showing the disposition of fibers in the opossum should be compared with these.

REMARKS ON THIS REGION IN THE AMPHIBIANS

In the writer's study of the selachian brain ('11) comparisons were made with the brain of the frog with reference to the position of the primordium hippocampi and its boundaries. At that time the writer accepted the sulcus limitans of Elliot Smith ('03) as the limit of the hippocampal formation and believed it to be the homologue of the sulcus which marks the boundary of the pallium in selachians. On this basis figure 75 of that paper was constructed. As has been stated elsewhere in this paper, the selachian sulcus is not the equivalent of the sulcus limitans of Smith in the reptile, while that in the frog is the same.

Anyone who will read the summary of errors and confusion in the literature of the amphibian septal region given by Elliot Smith ('03, p. 495) will understand that it would be useless for me to review this literature, especially as the result would be to note several additions to the list, made by later authors.

In the medial wall of the frog brain the sulcus and cell-free zona which have commanded the attention of all workers seems at once to divide the pallial from the basal areas and to correspond to the sulcus and zona limitans hippocampi in the selachian brain. It must be noticed, however, that a zona limitans, whether accompanied by a sulcus or not is usually the expression of a rearrangement of neurones in the adjacent areas due to functional or mechanical causes or both. In the selachian the zona limitans in the medial wall marks the line of meeting of simple olfactory with the olfacto-gustatory correlating centers. The line of demarcation between the two was perhaps determined by the connection of the neural tube with the ectoderm at the neuroporic recess. In the amphibian a second differentiation has begun and is well advanced in the frog, namely, the formation of hippocampus. This is marked by a change of form and rearrangement of cells in the pallium. The cells are now pyramidal and have their long axes placed vertically to the brain surface instead of being wholly without arrangement. Such a change constitutes a very marked difference from the selachian brain and leads to the formation of a new zona limitans. Below this zona limitans the neurones are scattered without any regularity. These facts, however, are not sufficient to show that the zona limitans separates pallial and basal areas. Immediately below the zona limitans appears a group of large cells which stand out conspicuously from the cells of the remainder of thisregion ('11 a, fig. 75, Herrick '10, fig. 40, *n. medialis septi*). When these cells are examined it is found that they form a column extending forward to the olfactory peduncle. This column of cells corresponds to the "septum" or primordium hippocampi of the turtle's brain. In the rather broad and shallow sulcus between it and the hippocampus runs the fimbria, as is well known.

Traced caudally this column of cells continues above the foramen interventriculare where it is separated from the developing hippocampus by a ventricular sulcus and a cell-free zone. This portion is known as the pars fimbrialis (Kappers) or the supraforaminal portion of the paraterminal body (Herrick). The fimbria runs in this body to be distributed to the posterior part of the hippocampal formation and in it the hippocampal commissure collects before it descends in the lateral wall behind the foramen. With the exception of the behavior of the hippocampal commissure the relations in the frog agree with those in the turtle. Behind the foramen it is not difficult to see that this body corresponds to the small primordium hippocampi in this position in the turtle and the sulcus along (beneath) which the fimbria runs is the fimbrio-dentate sulcus. Rostral to the foramen in the frog there is wanting the sulcus which separates the primordium hippocampi from the area parolfactoria in reptiles and mammals. Gaupp ('97, figs. 26, 28, 29) describes and figures a narrow cell-free zone between this nucleus and the cells below. He calls this column "ganglion septi," but it is entirely different from the nucleus septi of Unger, Kappers and others. The entire lower half of the medial wall has been assigned by most authors to the paraterminal body and it has been assumed that the paraterminal body extends up over the foramen.

Now this assumption is one of the most inexplicable things in the literature of forebrain morphology. It is quite possible to understand how the great growth and arching dorsad of the corpus callosum is mammals results in stretching up the commissure bed as set forth by Elliot Smith. And so long as it was thought that the commissure bed belonged below the neuroporic recess and was related to the lamina terminalis there was no escape from the logic of the argument so far as applied to mammals, that the paraterminal body had been stretched up into the supraforaminal position as the septum. But in reptiles and amphibians there is no such voluminous corpus callosum. There is therefore absolutely no motive for the extension of the paraterminal body up over the foramen. In amphibians there is not even a hippocampal commissure extending up over the foramen from in front. Moreover it is known that in reptiles at least the bed of the pallial commissures is supraneuroporic in origin. The supposition that a part of the paraterminal body has migrated into the caudal part of the hemisphere above the foramen is not only not supported by evidence but is quite unthinkable in view of all the known facts.

The writer would substitute for this view the following simple explanation of the septal region in the frog. Above and caudal to the foramen the medial wall is all pallial. A part of this pallial area has developed pyramidal cells and may be recognized as a true hippocampus in a low stage of organization. The remainder has retained the irregular arrangement of cells seen in the selachian pallium and gives passage to the fimbria and fornix fibers as in the reptile. Rostral to the foramen there has been the same development of hippocampus but the residue of hippocampal primordium is smaller and the line of demarcation between this and the area parolfactoria has been obscured in part. At the same time a new sulcus has developed in the medial surface. This sulcus forms along the line of demarcation between the pyramidal and irregular cells. The elongation of the hemisphere has led to the formation of the fimbria just below this sulcus, through the collection of many fibers of the olfacto-cortical system, on their way to the caudal part of the hippocampus. This sulcus is therefore the fimbrio-dentate sulcus.

Each element in the above explanation is perfectly simple and direct. There is no supposition that great masses of cells have migrated half the length of the hemisphere without motive. The only point of obscurity is the line of separation between the primordium hippocampi and the area parolfactoria. Since this is perfectly clear in reptiles and mammals it is quite legitimate to accept the hypothesis that in the frog it has lost somewhat in definiteness because of the small volume of the residual primordium hippocampi.

GENERAL OBSERVATIONS AND SUMMARY

When the results of the present series of studies are reviewed it is seen that great confusion prevails in the morphological conceptions and the nomenclature of this region of the brain. In these studies for the first time there has been built up a connected account of the evolution of the telencephalon beginning with primitive brains and taking into consideration the factors and processes by which the form of the mammalian telencephalon have been determined. Briefly summarized, these processes are the following: (a) The olfactory placode retards the closing of the neural tube, causing the formation of the anterior neuropore. The neuroporic recess, seen in most vertebrate brains, marks the dorsal or caudal border of this neuropore. (b) Early in vertebrate history the somatic sensory nerve of the telencephalic segment is greatly reduced or disappears while the olfactory nerve is very large in lower vertebrates. No motor nerve is present in this segment. (c) The olfactory fibers enter the visceral sensory column in the telencephalon and cause a great hypertrophy of this column, which rises up in the brain wall and pushes the somatic sensory column to the lateral surface ('11 a, p. 41; '11 b, p. 497, 513–517). (d) The hypertrophy of the visceral sensory columns together with the slight growth of ventral columns near the optic chiasma has produced a forebrain flexure such that the visceral sensory column takes the form of a letter U. The basal limb of the U is occupied by secondary olfactory centers, its dorsal limb adjacent to the diencephalon by the olfacto-gustatory correlation center ('12 b, p. 369). The formatio olfactoria is situated in the base of the U and receives the olfactory nerve. The space between the limbs of the U is filled by somatic sensory area. (e) The evagination of the hemisphere begins first at the formatio olfactoria, involves gradually the olfactory bulb and later the olfacto-gustatory correlation center and finally the somatic sensory area ('11 a, p. 42; '12 b, p. 363). The evagination does not modify the fundamental relations. (f) The absence of a primary somatic sensory nerve in the telencephalic segment left the somatic sensory column free to serve correlating functions for cutaneous, kinaesthetic, visual and other somatic impulses. This has been the controlling factor in the development of the general cortex ('10 b). The assumption of terrestrial life has led to the rapid development of this somatic area, and its expansion has pushed the secondary olfactory centers and olfacto-gustatory center into the positions occupied in mammals by the pyriform lobe and the hippocampal formation respectively. (g) Interrelations between the somatic area and the several regions of the visceral column have resulted in the development of special centers; from the larger part of the olfacto-gustatory center a hippocampus; from contiguous parts of the medial olfactory nucleus and somatic area, the corpus striatum; and from contiguous parts of the lateral olfactory nucleus and somatic area, the pyriform lobe. The details of all these processes remain yet to be worked out.

In the present paper we are concerned chiefly with the relations in the medial wall of the hemisphere and not with the general cortex or the pyriform lobe. The main questions at issue are the identification in reptiles and mammals of the basal olfactory center (medial olfactory nucleus or area parolfactoria) and the equivalent of the massive roof of the selachian telencephalon, the boundary line between the two, and the position of the forebrain commissures with relation to these two bodies. The result of our study has been to show:

1. That the neuroporic recess is situated just above the anterior commissure and below the anterior pallial commissure and between the pillars of the fornix when those structures are present. It is the recessus triangularis of Schwalbe and recessus inferior of Elliot Smith.

2. The boundary line between roof structures and area parolfactoria is marked in reptiles and many mammals by a groove running rostrad from the neuroporic recess in the ventricular surface of the medial wall and by a zona limitans in the structure of the wall. The zona limitans and ventricular sulcus are both the expression of functional differentiation of adjacent centers and indicate a rearrangement of the neurones in the gray matter adjacent to the ventricle.

3. The structures dorsal to this zona limitans include the hippocampal formation proper and the septum pellucidum or its equivalent in the brains of lower mammals and reptiles. These structures are all developed out of the roof of the selachian brain, called by the writer the primordium hippocampi.

4. The structures ventral to this zona limitans include a nucleus lateralis closely related to the head of the caudate nucleus, a nucleus medialis on the medial surface, islands of Calleja and the tuberculum olfactorium.

5. The hippocampal formation gradually differentiates a cortical layer in the medio-dorsal region of the hemisphere and, as

Elliot Smith and Levi have shown, the most medial border of this gives rise to the fascia dentata. Dorsal to the fascia dentata the hippocampus folds inward, on account of pressure from the expanding general cortex and perhaps other causes. This infolding produces the hippocampal fissure, which is known in embryos by the name fissura arcuata. This fissure is not present in the reptiles studied by the writer.

6. The septum pellucidum in lower mammals consists of a thick mass of gray matter which imbeds the fornix system and its commissure, becomes continuous with the hippocampal formation beneath the splenium of the corpus callosum and is connected with the supracallosal hippocampus by columns of cells between the bundles of the commissures. Rostrad the septum is continuous with the indusium around the genu corporis callosi and extends forward ventral to the precallosal hippocampus to the olfactory peduncle. This septum pellucidum represents a part of the roof of the selachian forebrain (primordium hippocampi) which has remained in a low stage of development. In this rostral continuation of the septum pellucidum there runs a longitudinal bundle of fibers which are collected from the basal olfactory centers and reach the hippocampus either through the fimbria or by way of the stria Lancisii. Between this fimbria-bundle and the fascia dentata a sulcus appears in reptiles and mammals which is the fimbrio-dentate sulcus of human anatomy.

7. The use of the term primordium hippocampi in the sense which is given to it in this paper requires some comment. The term is used by me to denote that lowly organized pallial mass in the selachian brain from part of which the hippocampal formation has developed. It is also used for the homologous mass in cyclostomes where it is situated in the telencephalon medium. It is also used for the residue which is left from this mass in higher vertebrates after the hippocampal formation has been developed. This residue is the body long known as the septum pellucidum. The hippocampal formation and septum together constitute a continuous mass of gray matter which thickens the lamina supraneuroporica and forms the bed for the hippocampal commissure and corpus callosum.

The term primordium hippocampi was first used by Elliot Smith ('03) to designate that portion of the reptilian and amphibian hemisphere which corresponds to the mammalian hippocampal formation. When the writer realized that the body in the brain of fishes which he had called 'epistriatum' was in reality the forerunner of the hippocampus, he adopted the name primordium hippocampi. At that time the writer supposed that his primordium hippocampi in fishes was approximately equivalent to Elliot Smith's primordium hippocampi in reptiles. It now appears that the primordium hippocampi of the writer includes the body to which Elliot Smith gave the same name plus the equivalent of the septum.

The most important matter is that the exact meaning of terms be understood. Upon the lesser question as to what terms are most appropriate a few words may be said. Elliot Smith's primordium hippocampi is the equivalent of the hippocampus and fascia dentata. In the reptilian brain it is bounded by a sulcus which he called sulcus limitans hippocampi. This name is literally appropriate and it is now clear that the sulcus in selachians to which the writer applied the same name is an entirely different sulcus. The sulcus limitans hippocampi of the frog as recognized by Herrick and the writer is the same as that in reptiles. It is shown in this paper that this sulcus lies between the fascia dentata and the fimbria. Since the term sulcus fimbrio-dentatus is in common use and clearly understood in descriptions of the human brain, it can be used for this sulcus in reptiles and amphibians and the term sulcus limitans becomes unnecessary. There is, however, need for a term to designate the boundary between the pallial and sub-pallial areas in the medial wall. This is what Elliot Smith attempted to do by his term sulcus limitans. It is to this sulcus which limits the pallium that the writer has applied the name sulcus limitans hippocampi. This term is inappropriate because of the extreme divergence in structure between the hippocampus and septum in mammals, although they are indistinguishable in selachians, and remain similar in function. What is needed is some term to indicate that the septum belongs to the pallial area. This suggests such terms as sulcus marginalis pallii or fovea limbica medialis, or sulcus rhinalis medialis. Since the term sulcus rhinalis (lateralis) is used for the sulcus which marks the boundary between the pallium and the olfactory area laterally, the term sulcus rhinalis medialis seems peculiarly appropriate for this sulcus in the medial wall. In other words, the writer having used the term sulcus limitans hippocampi throughout this paper in the same sense as in former papers, would now substitute for it the term sulcus rhinalis medialis. The term sulcus limitans hippocampi of Elliot Smith is synonymous with the term sulcus fimbrio-dentatus.

As for the term primordium hippocampi, the writer believes that it is best to retain this to include the equivalent of hippocampal formation plus septum pellucidum. Two considerations strongly support this. The one is that the two are undivided and indistinguishable in fishes, the other is that the fimbria is usually considered to be an integral part of the hippocampal formation but is separated from Elliot Smith's primordium hippocampi by his sulcus limitans.

8. The hippocampal commissure and corpus callosum correspond in all their morphological relations to the two commissures in the roof of the telencephalon in selachians which are related respectively to the primordium hippocampi and the somatic sensory area. The marsupial Didelphys possesses true corpus callosum fibers running in the dorsal forebrain commissure. This is apparently the typical condition in selachians, marsupials and mammals. A corpus callosum has not been certainly demonstrated in reptiles.

9. The development by Elliot Smith of the idea that the pallial commissures are originally imbedded in the sub-pallial paraterminal body and that the great development and arching up of the corpus callosum has stretched and raised up the paraterminal body to form the septum pellucidum, has led to the recognition of a large body situated above the foramen of Monro which, although apparently in a pallial position, has had a sub-pallial origin. This is the body known in the work of recent writers as the supraforaminal portion of the paraterminal body. This body in lizards not only forms a bed for the anterior pallial com-

missure but extends back in the medial wall of the hemisphere beneath the hippocampus to the posterior pole, where it forms the bed for the posterior pallial commissure also (Elliot Smith, '03, Herrick '10).

In the light of the whole content of the present paper it is obvious that the writer would regard the supraforaminal mass in question as not belonging to the paraterminal body at all but to the pallium. It is without question derived from the pallium of the selachian brain and is related to the lamina supraneuroporica and not to the lamina terminalis. The term paraterminal body should be restricted to the basal olfactory centers in the medial wall which are in relation with the lamina terminalis. This body never reaches above the foramen to any significant extent. The writer has recognized a small projection of the paraterminal body above the foramen in selachians but it is of no importance. Nearly the same condition exists in the mole.

The general relations of the gray masses below the neuroporic recess and the zona limitans are clear. Although we by no means understand all the factors which have called forth special collections of neurones in this region, we may say that they all belong to the medial portion of the olfactory lobe or the medial olfactory area. The tuberculum olfactorium is a basal nucleus related at its two borders with the lateral and medial olfactory nuclei. It consists of a deeper, more compact, layer containing islands of Calleja and of a superficial layer of loosely scattered cells. Both these layers are continued into the medial wall where the superficial cells form a broad thin plate on the medial surface. Between the tuberculum and the ventricle is the massive head of the caudate nucleus and this extends around the ventral angle of the ventricle to form the deep layer of the medial wall. There are, therefore, superficial, middle and deep layers of cells in the medial wall. The several cell aggregates have been designated by various authors by such names as nucleus septi, nucleus accumbens septi, nucleus medianus septi, and so forth.

The term 'septum' has been applied to two independent structures in the telencephalon, the septum pellucidum of higher mammals and the medial olfactory nucleus or area in the medial wall of the brain of reptiles and amphibians. It has been the belief of most authors that this so-called septum in amphibians and reptiles is the equivalent of or is intimately associated with the septum pellucidum of mammals. In reality it includes both the medial olfactory nucleus and the equivalent of the septum pellucidum of mammals. Adolf Meyer ('92) and Unger ('06) distinguished the septum pellucidum as an independent structure in reptiles. The terms 'nucleus septi,' 'nucleus lateralis septi,' and so forth, are confusing and require revision. The nucleus septi (Unger; nucleus accumbens septi, Kappers) is said by these authors to belong to the striatum and not to the septum. It is divided by Herrick (fig. 43) into nucleus accumbens septi and nucleus lateralis septi. The latter nucleus includes in addition a great part of the equivalent of the septum pellucidum (figs. 47, 61, 56, 64, 66). The term 'nucleus medianus septi' is used by Herrick for part of the primordium hippocampi (figs. 43, 61), and also for the superficial layer of cells in the area parolfactoria (fig. 66). In other words, the lateral and medial nuclei 'of the septum' each contains a part of the parolfactory and a part of the pallial areas. Similar inconsistencies appear in the work of other authors. Generally speaking, recent authors have followed Elliot Smith in regarding the septum of mammals as a derivative of the paraterminal body which is basal. They have consequently applied the term septum to the basal part of the medial wall in reptiles and amphibians. In this way the term septum has been made to include both basal olfactory and pallial areas. For this reason all reference to the septum should be eliminated from the names hereafter used for the several nuclei belonging to the basal olfactory areas. With this in view I have adopted the term, area parolfactoria, and have called the superficial layer of cells the nucleus parolfactorius medialis and the deep layer which is so closely related to the caudate, the nucleus parolfactorius lateralis. It should be noted that this area corresponds nearly to the area parolfactoria of Broca, and that the writer regards as very unfortunate the recent use of the term to designate the tuberculum olfactorium or an adjacent part of the anterior perforated space (lobus parolfactorius, Edniger '08, p. 260; eminenza paraolfattoria, Beccari '11). A discussion of the relations and terminology of the region surrounding the tuberculum olfactorium must be reserved for another time.

The general results of the foregoing study should be summarized lest the interest in various details should overshadow the main significance of such a comparative study. The reptilian and mammalian equivalents of the pallial and basal areas of the selachian telencephalon and the boundary between them have been accurately defined so far as the medial wall is concerned. It has been shown that the septum pellucidum or its equivalent is a derivative not of the basal olfactory area but of the pallial area. It is the unchanged residuum of the selachian pallium after the hippocampal formation is developed. The fusion of the two septa (hippocampal primordia) in the lamina supraneuroporica forms the bed for the passage of the pallial commissures between the hemispheres. It is shown that the two commissures in the selachian pallium hold essentially the same relations to all structures in the median region as are held by the hippocampal commissure and corpus callosum in mammals. It is therefore strongly probable that the commissure of the somatic area in the selachian telencephalon is the true forerunner of the mammalian corpus callosum. The final determination whether this is true or not will come with the further study of the history of the somatic area and the clear demonstration of corpus callosum fibers in reptiles and dipnoi.

The writer wishes to correct here, with due apology to the authors concerned, an unfortunate slip made in an earlier publication. In the paper on the selachian brain ('11 a, p. 58, sec. 40) occurs a sentence which should read as follows: "The terms neopallium (Elliot Smith) and archipallium (Edinger) are therefore not appropriate." I was acquainted with Professor Smith's disclaimer of the term archipallium (Anat. Anz., Bd. 35, p. 429) and it was my express intention in the sentence quoted to credit the two terms to their proper authors. Through some inexplicable error the names became interchanged and the mistake was not noticed until recently.

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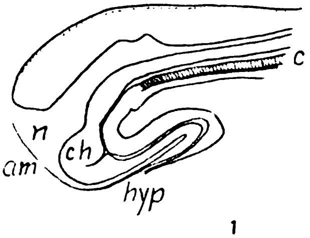
REFERENCE LETTERS

a.h., association bundles of the hippocampus
alv., alveus
am., amnion
a.t., angulus terminalis
b.o., bulbus olfactorius
c., chorda dorsalis
c.a., commissura anterior; also the commissural bundle of the olfactory tract
cbl., cerebellum
c.c., corpus callosum
c.d., cortex dorsalis
c.e., capsula externa
c.f., columna fonicis
cg., cingulum
c.h., commissura hippocampi
c.i., capula interna
ch.op., chiasma opticum
c.l., cortex lateralis
c.m., corpus mammillare
c.post., commissura posterior
c.p.a., commissura pallii anterior
c.p.p., commissura pallii posterior
c.r., corona radiata
c.s., commissura superior
c.st., corpus striatum
dec.po., decussatio postoptica
dienc., diencephalon
e., epiphysis, pineal body
em.th., eminentia thalami
ep., epistriatum
f., fornix
fasc.m., fasciculus marginalis
f.pc., fasciculus praecommissuralis
f.d., fascia dentata
f.sag., fissura sagittalis
fi., fimbria
flex.h., hippocampal flexure
f.o., formatio olfactoria
for.i., foramen interventriculare
f.rh., fissura rhinalis
f.s., fornix superior
g., genu corporis callosi
h. hippocampus
hab., nucleus habenulae
hem., hemisphere
hy., hypothalamus
hyp., hypophysis
i., indusium
i.C., islands of Calleja
l.pyr., lobus pyriformis
l.s., lamina supraneuroporica
l.t., lamina terminalis
m., margo posterior pallii, caudal margin of the lamina supraneuroporica
n., neuropore
n.c., nucleus caudatus
n.m., nodulus marginalis
n.o.a., nucleus olfactorius anterior
n.p.l., nucleus parolfactorius lateralis
n.p.m., nucleus parolfactorius medialis
n.t., nervus terminalis
n.tr.olf.lat., nucleus of the lateral olfactory tract
p., paraphysis
para., paraterminal body
p.f., perforating fibers of fornix superior
p.h., primordium hippocampi
r.c.c., rostrum corporis callosi
r.i., recessus infundibuli
r.p., recessus praeopticus
r.po., recessus postopticus
r.m., recessus mammillaris
r.n., recessus neuroporicus
r.n.e., recessus neuroporicus externus
r.s., recessus superior
s.c.c., sulcus corporis callosi
s.d., saccus dorsalis
s.en., sulcus endorhinalis
s.f-d., sulcus fimbrio-dentatus
s.hy., sulcus hypothalamicus (sulcus Monroi)
s.l., sulcus limitans hippocampi
s.l.H., sulcus limitans of His
s.l.L., stria lateralis Lancisii
s.m., stria medullaris
s.m.L., stria medialis Lancisii
s.o., sulcus olfactorius

spl., splenium
s.t., stria terminalis
sub., subiculum cornu ammonis
t.c., tela chorioidea
t.f., taenia fornicis
thal., thalamus
th.r., thalamic radiations
t.o., tuberculum olfactorium
t.p., tuberculum posterius
tr.olf., tractus olfactorius
tr.op., tractus opticus
v.l., ventriculus lateralis
v.tr., velum transversum
z.l. zona limitans



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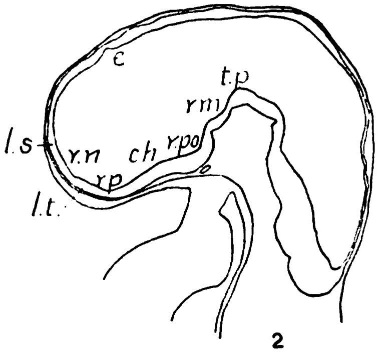

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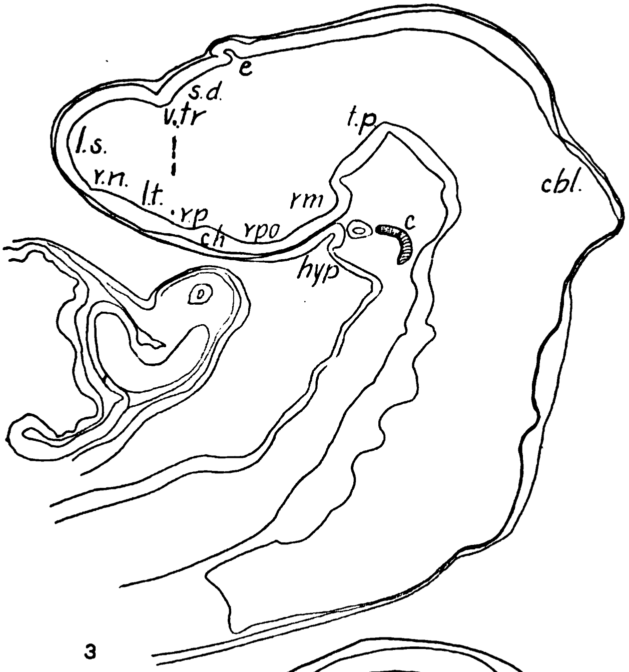
Fig. 1 Chelydra serpentina, 2 mm. embryo, median sagittal section of the anterior portion to show the neuropore and adjacent structures. Magn. 52 diam.

Fig. 2 Chelydra serpentina, 4.5 mm. embryo, sagittal section of head to show neuroporic recess, lamina terminalis and lamina supraneuroporica. Magn. 52 diam. The section is median in the anterior region.



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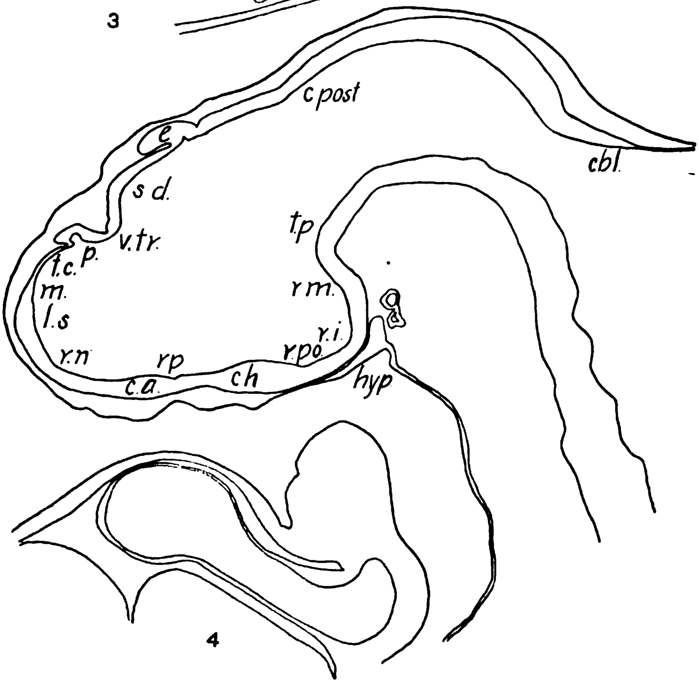

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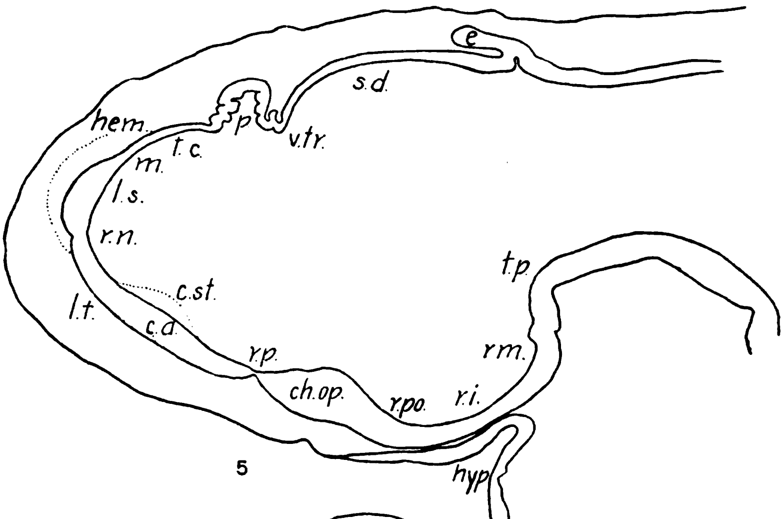
Fig. 3 Chelydra serpentina, 6 mm. embryo, median sagittal section. Magn. 52 diam.

Fig. 4 Chelydra serpentina, 9 mm. embryo, median sagittal section. Magn. 52 diam.



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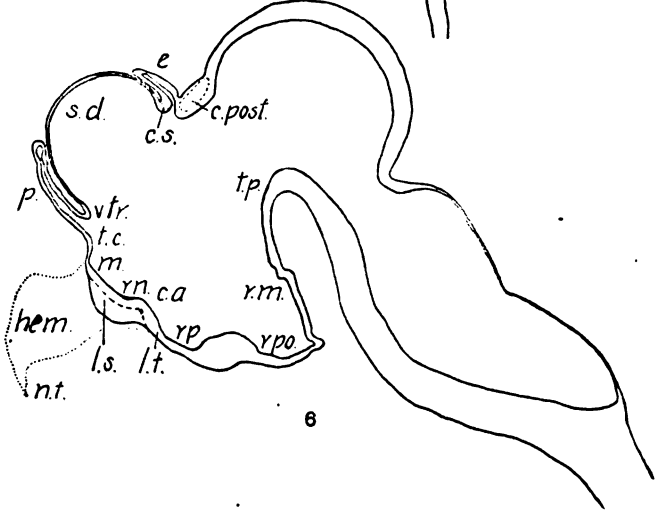

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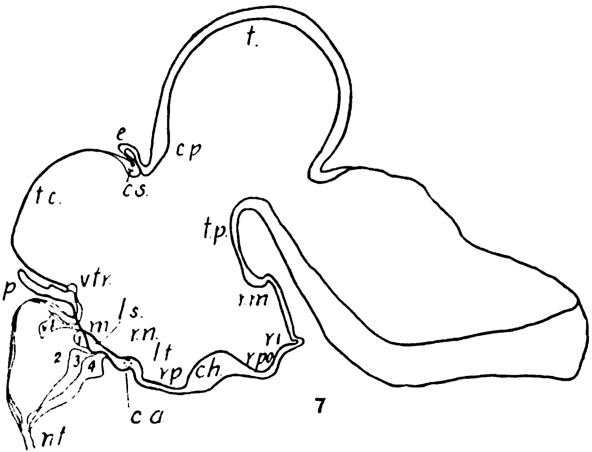
Fig. 5 Chelydra serpentina, 10 mm. embryo, median sagittal section. Magn. 52 diam. In the 6, 9 and 10 mm. stages the lamina supraneuroporica grows in thickness. The point of transition to the tela is the margo posterior pallii. The fibers of the anterior commissure are evident in this stage.

Fig. 6 Chelydra serpentina, median sagittal section of the brain of a specimen having a carapace 8 mm. in length. Magn. 17 diam. The thickening of the lamina supraneuroporica now begins to encroach upon the recessus neuroporicus, which is nearly obliterated in the adult.



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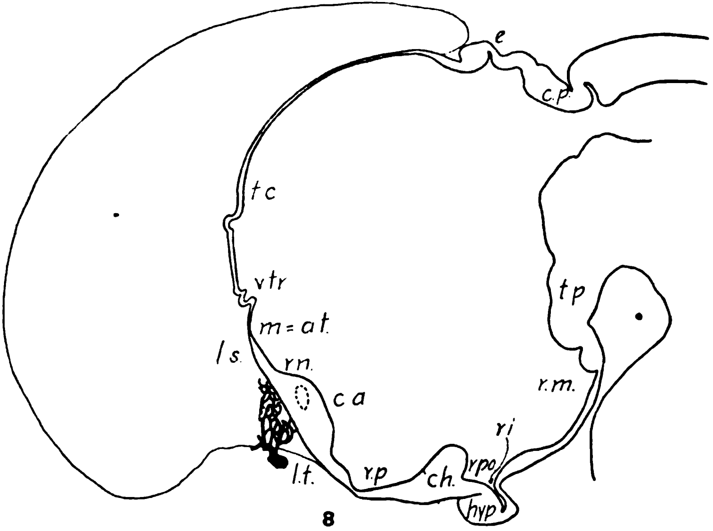

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Fig. 7 Emys lutaria, median sagittal section of the brain of an embryo slightly more advanced than that shown in figure 6.

Fig. 8 Human embryo, 31 mm. C. R. length, median sagittal section of brain. Huber collection, No. XLVII. In the relations about the neuroporic recess this embryo agrees essentially with the turtle embryos.

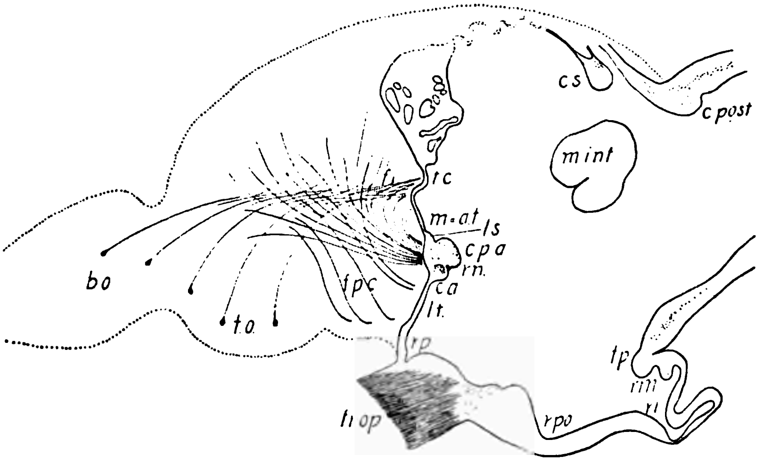
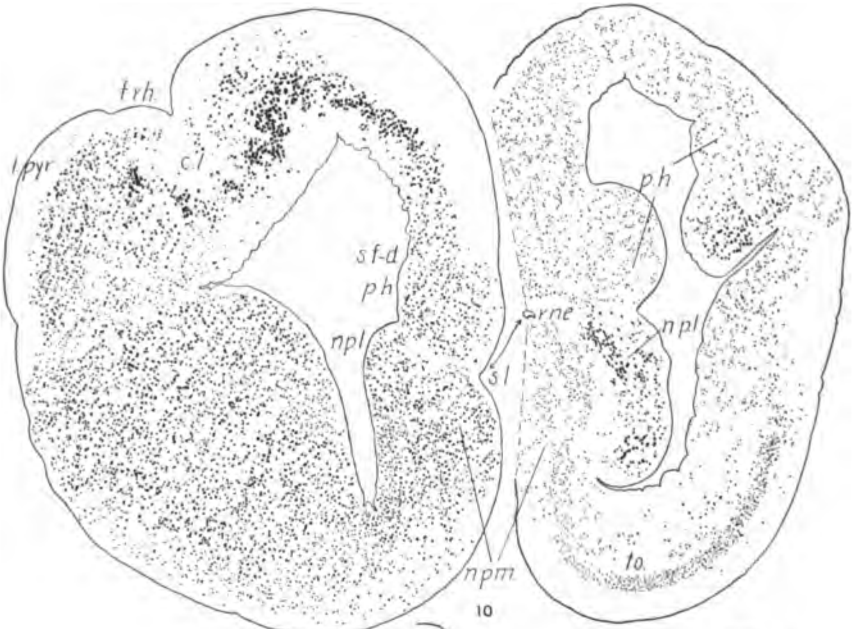


Fig. 9 Cistudo carolina, adult, median sagittal section of forebrain. The pallial commissure has descended into close relation with the anterior commissure so that the neuroporic recess is nearly obliterated. The median section is accurately reconstructed from sagittal sections. The dotted outline of the hemisphere is diagrammatic. For the explanation of the fibers shown, see text.



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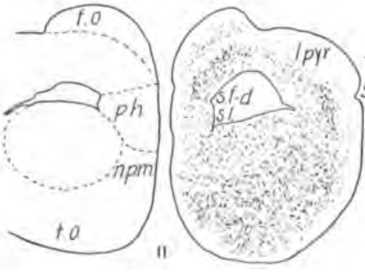

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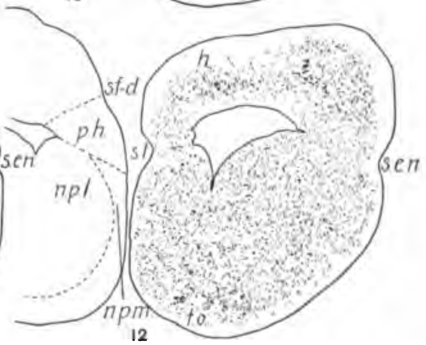

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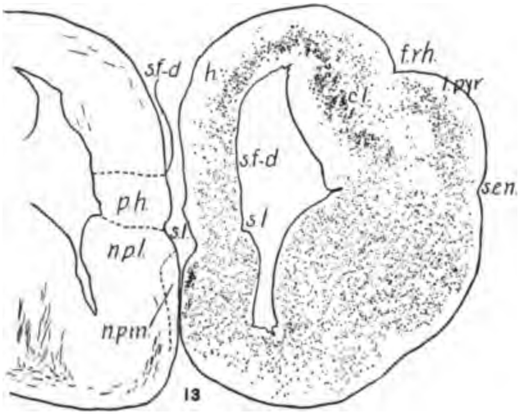
Fig. 10 A figure for the comparison of the selachian brain with that of the turtle. The right half of the figure represents a section of the brain of Acanthias (from '11 a, fig. 75). The left half represents a section at a corresponding level of the brain of the turtle. Both drawings were made under the Edinger apparatus and the grouping of cells is accurately represented. At this level in the brain of Acanthias the nucleus parolfactorius lateralis appears to be confined to the medial wall, but farther rostrad it has a broad connection with the deep gray of the lateral wall quite as in mammals. It does not appear that this nucleus represents an invasion of the medial walls by the striatum. It is rather a primary component of the medial wall.



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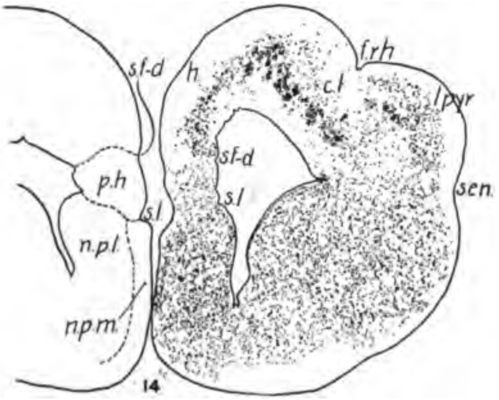

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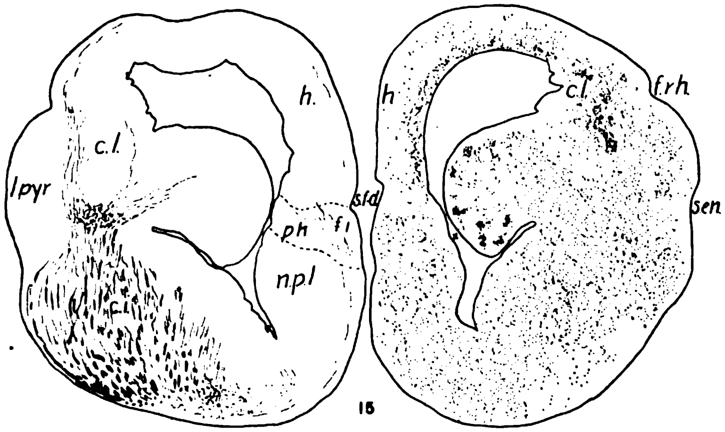
Figs. 11 to 18 Cistudo carolina. Eight transverse sections of the hemispheres. The right half of each section is drawn from a series stained with a cell stain, the left half from a Weigert series. Owing to variation between individuals and to slight differences in the plane of section the outlines of the two halves do not agree perfectly, but the right and left sides are substantially equivalent. The drawings were made under the Edinger apparatus. On the right side each cell is represented by an ink dot. The number and grouping of the cells are accurately shown, the relative size fairly well. Magn. 13 diam.

Fig. 11 Section through the olfactory peduncle and the caudal border of the formatio olfactoria. It shows the two ventricular sulci separating from one another in front of the peduncle (compare fig. 19). Between the two is the primordium hippocampi and above this the rostral end of the hippocampus.

Fig. 12 Section in the caudal part of the peduncle showing the two ventricular sulci close together.

Figs. 13 and 14 Successive sections farther caudad. The sulcus limitans between the area parolfactoria and the primordium is better marked than that (*s. f-d.*) between the primordium and hippocampus.



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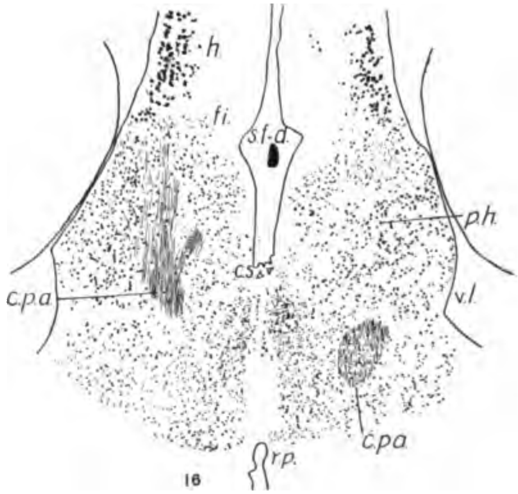

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Fig. 15 Section at about the level where the sulcus limitans bends downward and is less conspicuous in transverse sections. It is marked by a broken line on the left and on the right by a small cross.

Fig. 16 A section taken just at the rostral border of the superior recess. The section is magnified 28 diameters. The anterior pallial commissure rises at either side in the hippocampal primordium. The two dense nuclei of small cells near the middle line are in the rostral wall of the superior recess and correspond in part to the nodulus marginalis of the bat and rodents. A part of this nucleus is represented also by the small-celled nucleus adjacent to the hippocampal commissure (e.g., in figs. 46 and 64). *c. s.*, recessus superior.

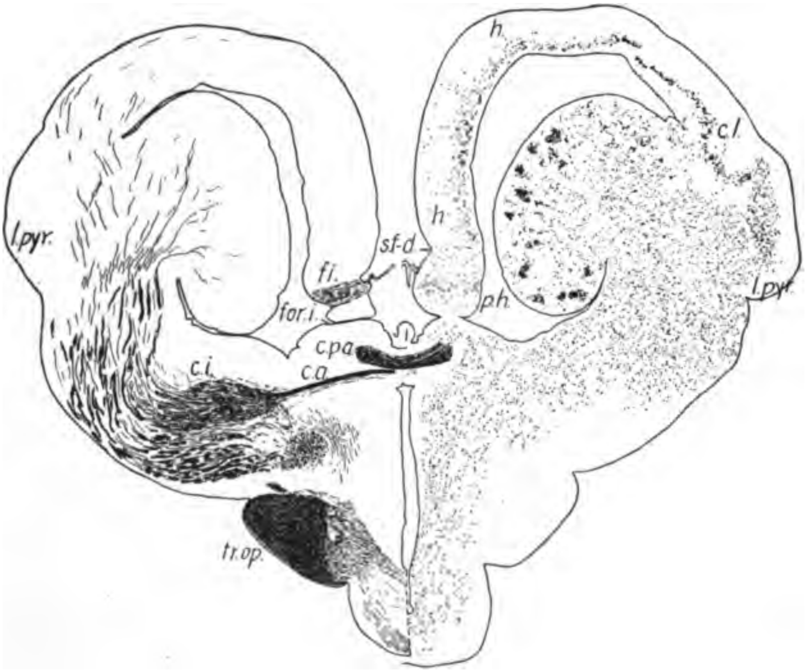


Fig. 17 Section at the rostral border of the interventricular foramen passing through the commissures. At this level the hippocampal primordium rapidly decreases in size, as seen on the left.

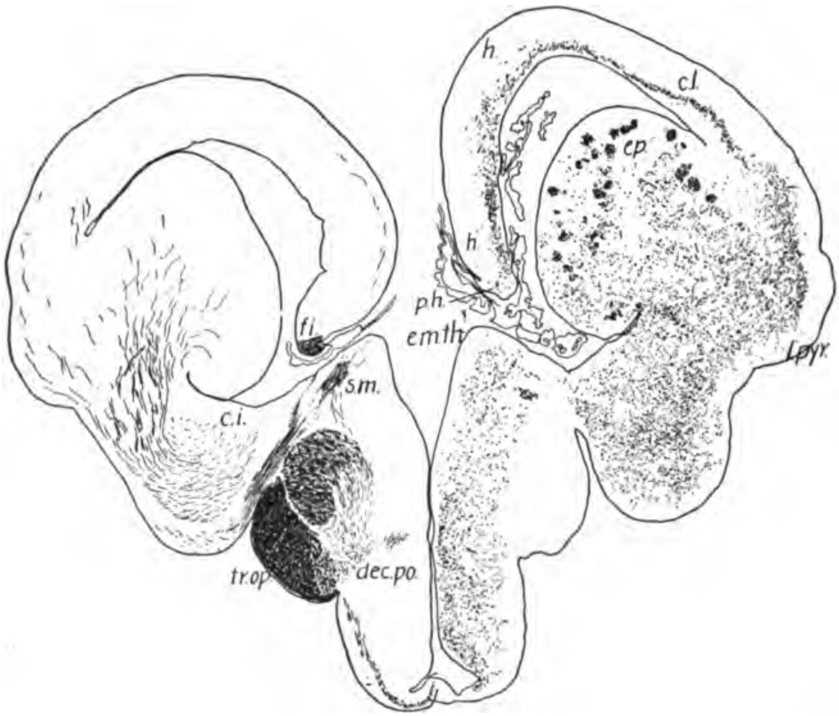
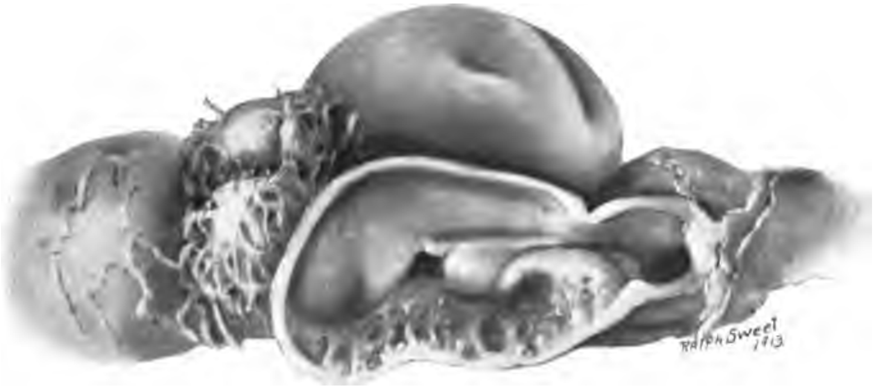


Fig. 18 Section near the caudal border of the foramen. The primordium is small here and is nearly filled by the fibers of the fimbria.



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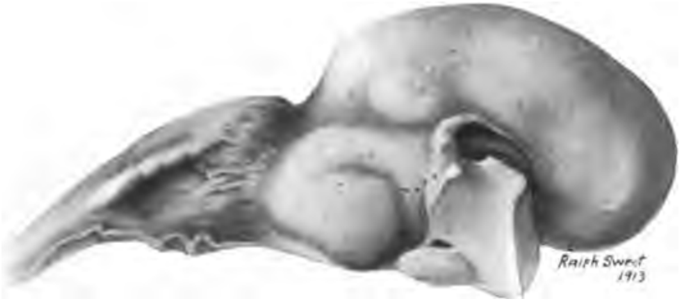

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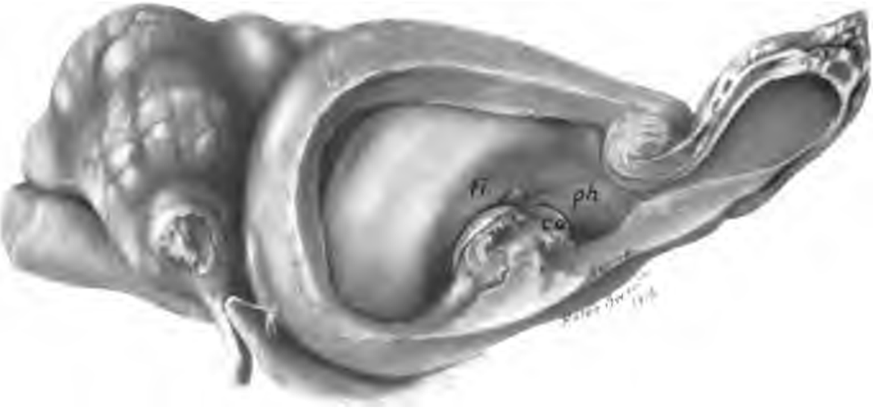
Fig. 19 Cistudo carolina, dissection of the right hemisphere to show the medial wall through the lateral ventricle. Nearly the whole lateral wall of the hemisphere and the choroid plexus have been removed. The rough surface in the lower part represents the region in which the large fiber bundles were dissected away by means of needles. The area parolfactoria appears as a somewhat bean-shaped ridge. Between this and the foramen is the greater part of the primordium hippocampi. It extends forward as a very slender ridge in the groove over the area parolfactoria and becomes enlarged in the olfactory bulb. Compare figures 18 and 19. The light ridge over the primordium is occupied by the fimbria.

Fig. 20 Cistudo carolina, medial view of the right hemisphere after removal of the brain stem. Note especially the slender primordium hippocampi and the fimbrio-dentate sulcus extending into the temporal region of the hemisphere. In lizards the primordium is much larger.



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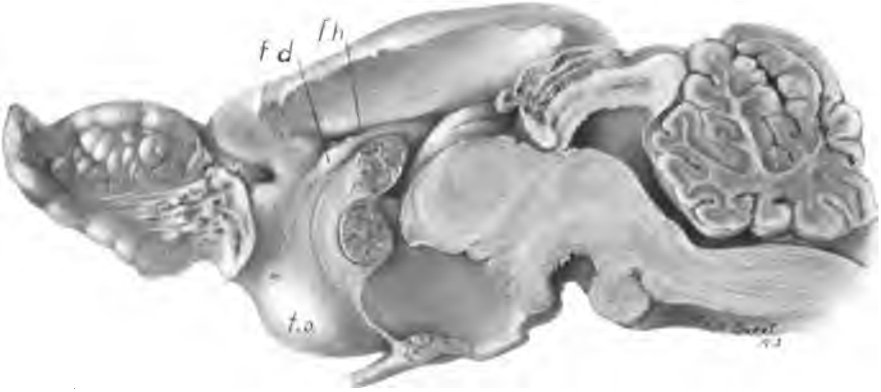

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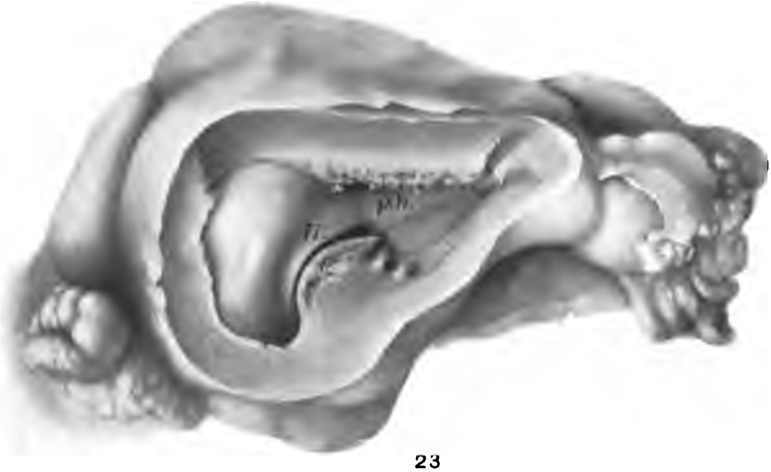
Fig. 21 Opossum (Didelphys virginiana). Dissection of the right hemisphere, as in figure 19. The area parolfactoria is relatively much smaller than in the turtle. The sulcus limitans is nearly horizontal in position.

Fig. 22 Opossum, medial view of the right half of the same brain as shown in figure 21. Description in the text.

Fig. 23. Striped gopher (Spermophilus tridecemlineatus). Dissection of right hemisphere as in figure 19. The sulcus limitans runs straight forward from the foramen. Note how the fimbria continues directly caudad from the primordium hippocampi.



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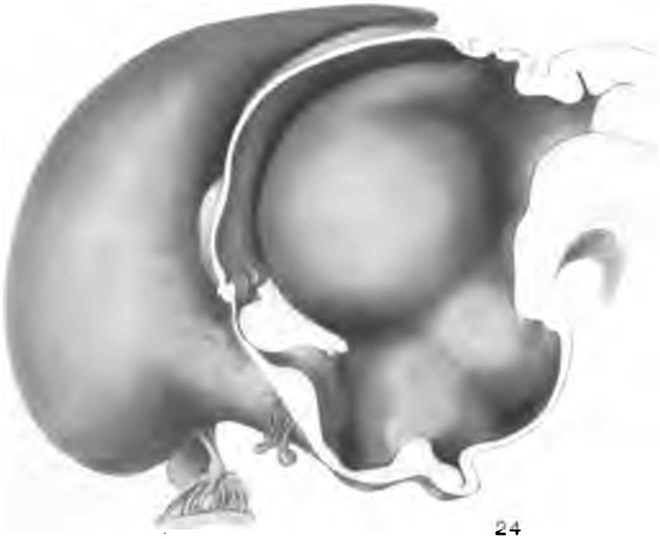

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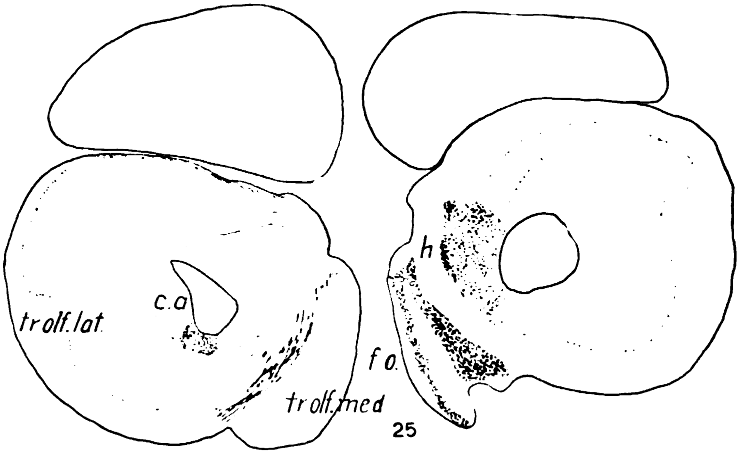
Fig. 24 Human embryo of 31 mm. Huber collection No. XLVII. Model of the medial portion of the right half of the brain. For reference letters, see figure 8. The model was made from sagittal sections for the purpose of reconstructing the median plane and the structures about the neuroporic recess. Not enough sections were included in the model to give the complete boundary of the interventricular foramen. The recessus neuroporicus is between *l.t.* and *l.s.* The blood vessels related to it are better drawn in figure 8. Note the well-marked sulcus hypothalamicus which crosses the sulcus limitans of His to form the sulcus Monroi.



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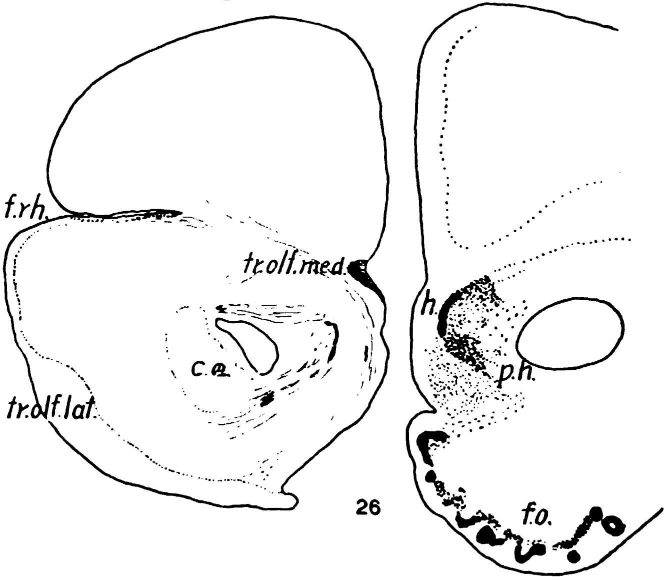

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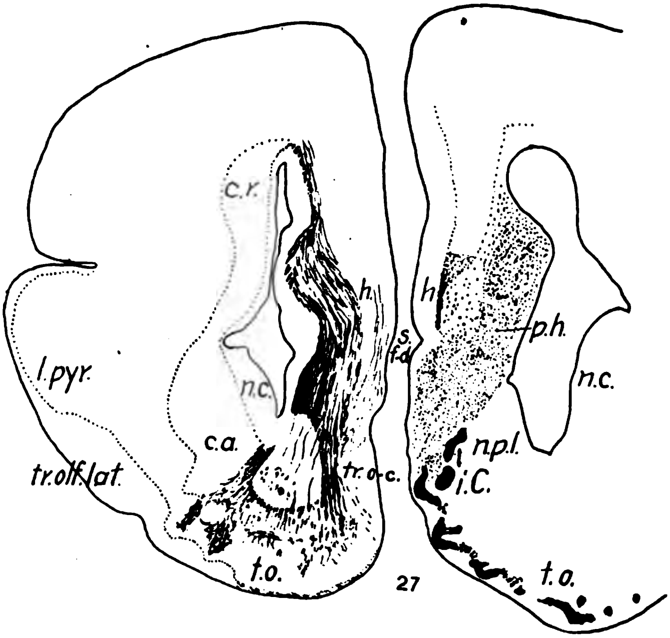

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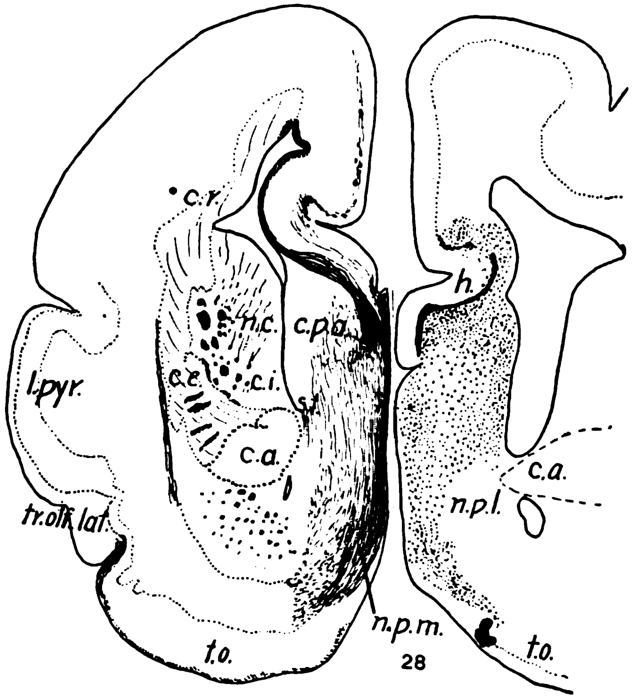
Figs. 25 to 30 Opossum, a series of transverse sections to show the relations of structures in the medial wall of the hemisphere. The right half of each figure is drawn from a series of cell-preparations, the left half from a Weigert series. On the right side the cells of the hippocampal formation and adjacent structures are drawn under the Edinger apparatus. The magnification (10 diam.) is too low to allow the form or size of the individual cells to be represented in most cases. The grouping is accurate. On the left side are drawn the fiber tracts of the medial wall.

Fig. 25 Opossum, transverse section through the olfactory peduncle. Description in the text.

Fig. 26 Opossum, transverse section just caudal to the olfactory peduncle. For the destination of the medial olfactory tract, see figure 35. Note the mass of large cells in the primordium hippocampi.

Fig. 27 Opossum, transverse section through the rostral end of the caudate nucleus and olfactory tubercle. The olfacto-cortical fibers arising from the tuberculum curve around the rostral surface of the nucleus parolfactorius lateralis, and appear to be interrupted in this section. Note the great extent of the primordium hippocampi.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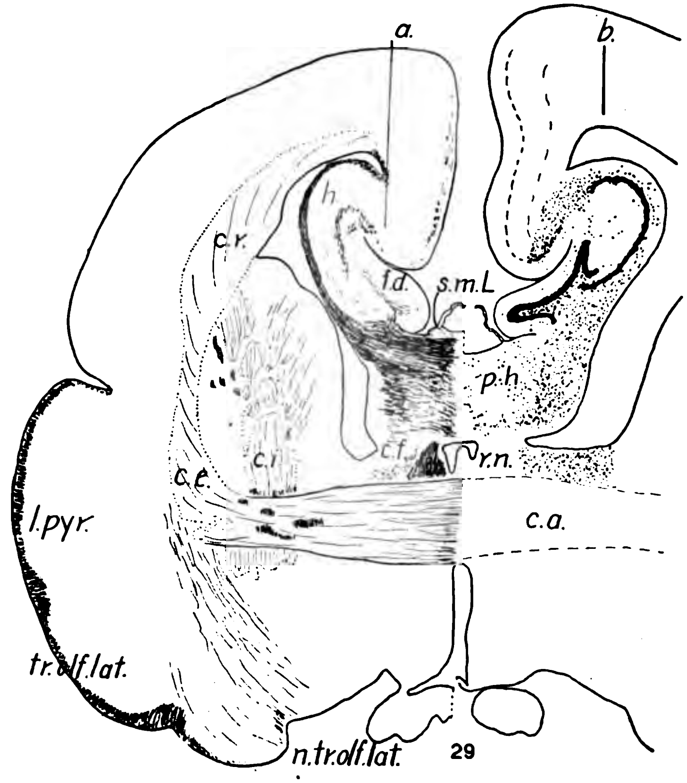
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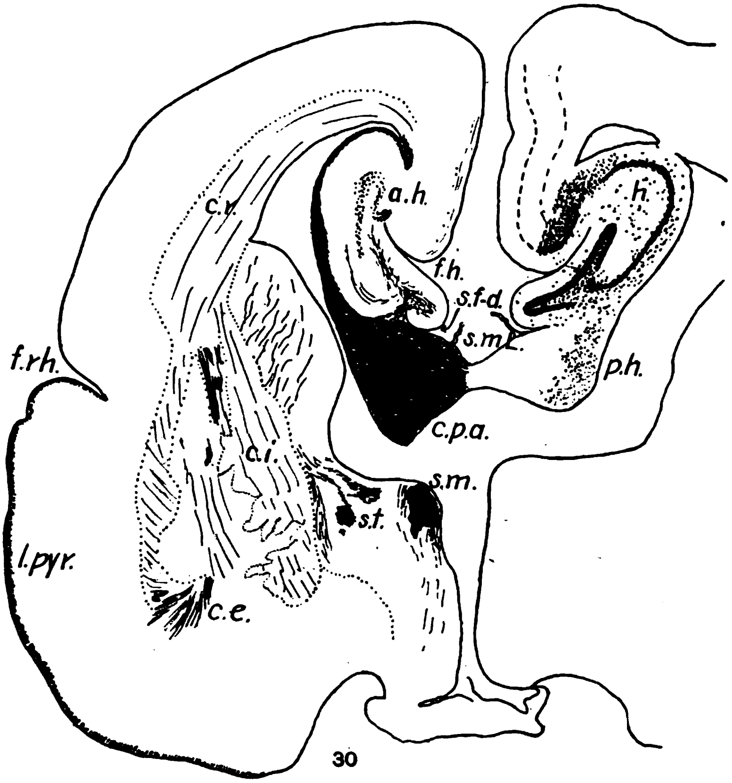
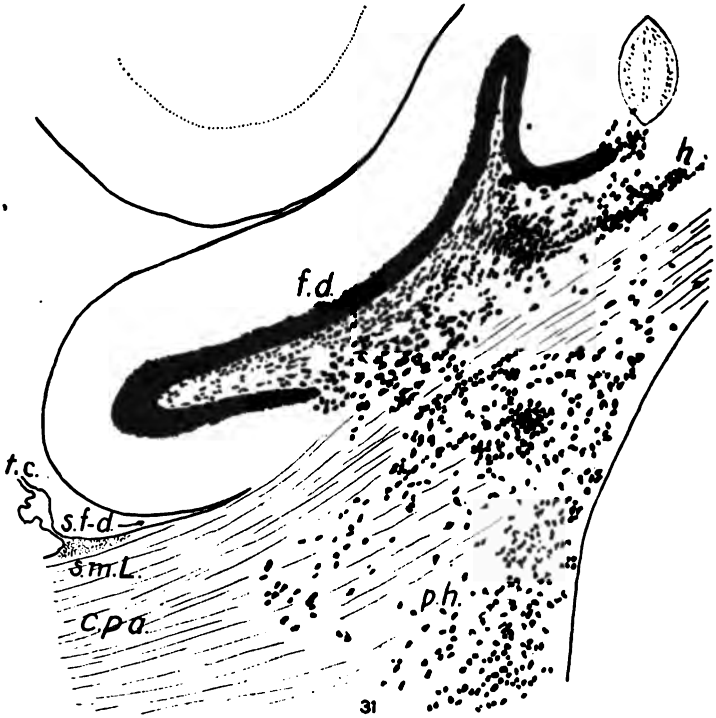


Fig. 28 Opossum, transverse section a short distance rostral to the anterior commissure. Note that the nucleus parolfactorius medialis is imbedded in the precommissural bundle. Note the continuity of the fascia dentata and the hippocampus and the close connection of the primordium with both. The rostral limb of the anterior pallial commissure contributes most of the fibers to the alveus at this level.

Fig. 29 Opossum, transverse section through the neuroporic recess. Here is seen the typical fully developed hippocampal formation with the primordium transversed by the anterior pallial commissure. All above the neuroporic recess belongs to the pallium. The commissure was thicker in the Weigert specimen than in the other. For the stria medialis Lancisii compare figure 37.

Fig. 30. Opossum, transverse section through the interventricular foramen and at the caudal border of the anterior pallial commissure. The fusion of the hippocampus with the overlying dorsal wall seen in the right half of the figure occurs frequently. In these fused places fibers seem to pass from the corona radiata into the alveus. These would probably be regarded as corpus callosum fibers. Of the three bundles constituting the stria terminalis the middle one enters the anterior commissure, the lower one passes beneath the anterior commissure, and the upper one passes over the anterior commissure and down in front of it.



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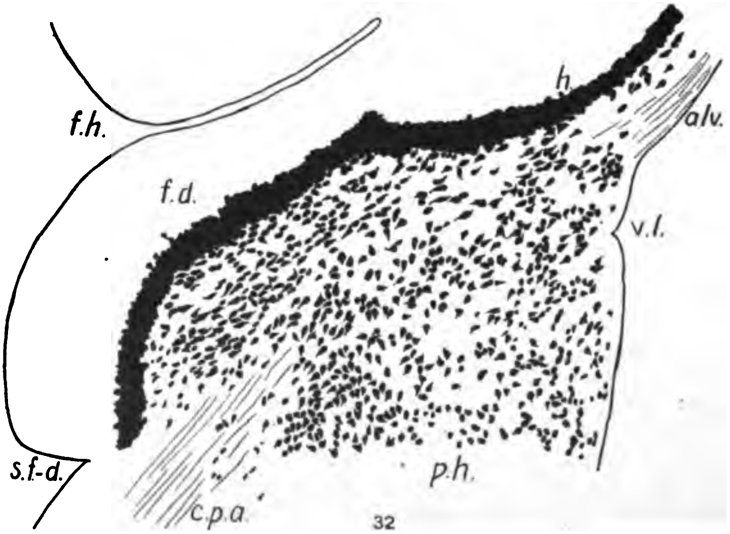

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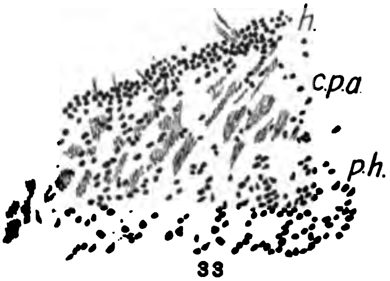

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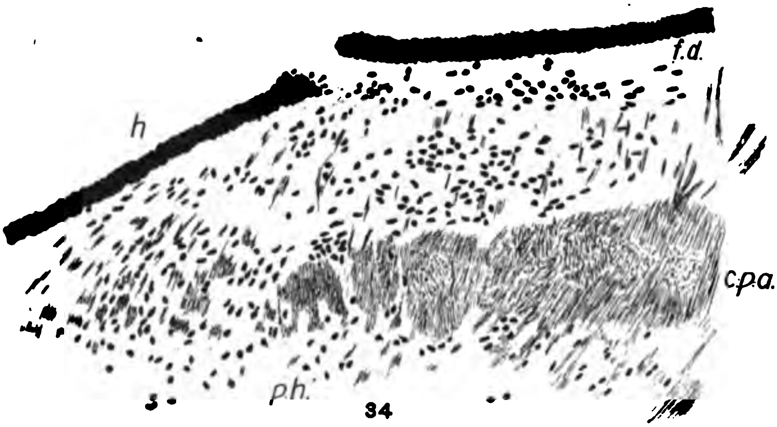

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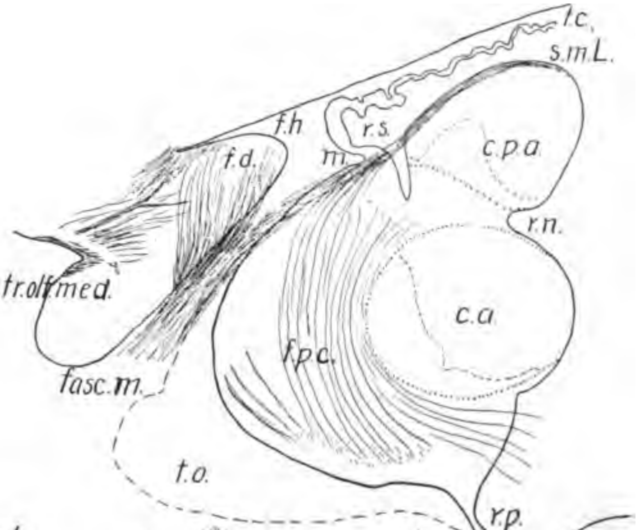
Figs. 31 and 32 Opossum, two transverse sections through the hippocampal formation to show the relations of the primordium. Cell preparation. Description in text.

Fig. 33 Opossum, part of a parasagittal section cutting the rostral limb of the anterior pallial commissure. Its position is indicated by the line *b* in figure 29.

Fig. 34 Similar figure to the last, from another specimen. Section nearer the median plane (line *a*, fig. 29). Both figures show that the commissure fibers as they pierce the wall to reach the alveus are arranged in larger and smaller bundles and that between these a considerable amount of space is filled with cells which establish continuity between the primordium hippocampi and the polymorphic layer of the hippocampus. Magn. 60 diam.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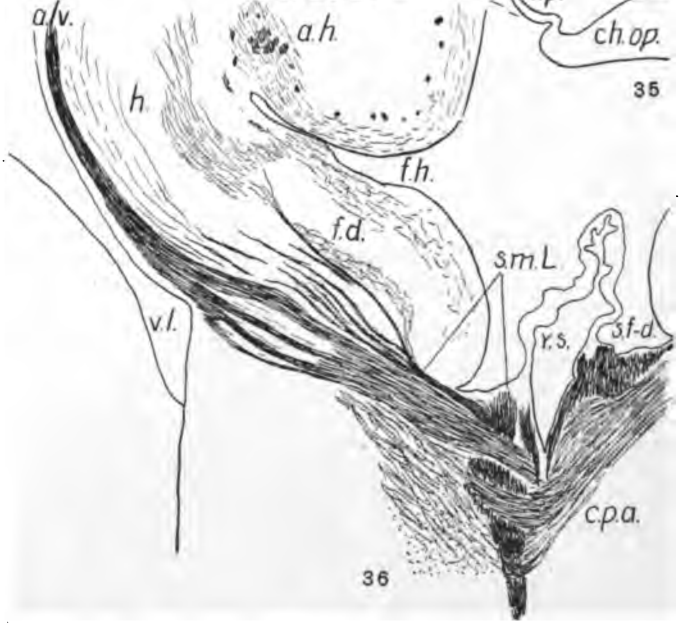
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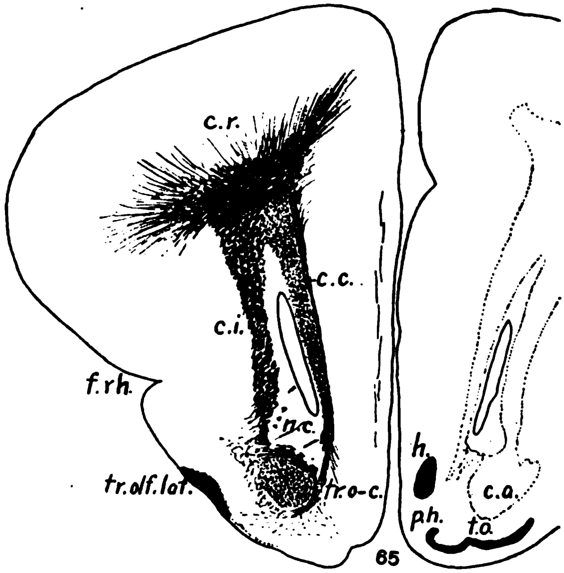
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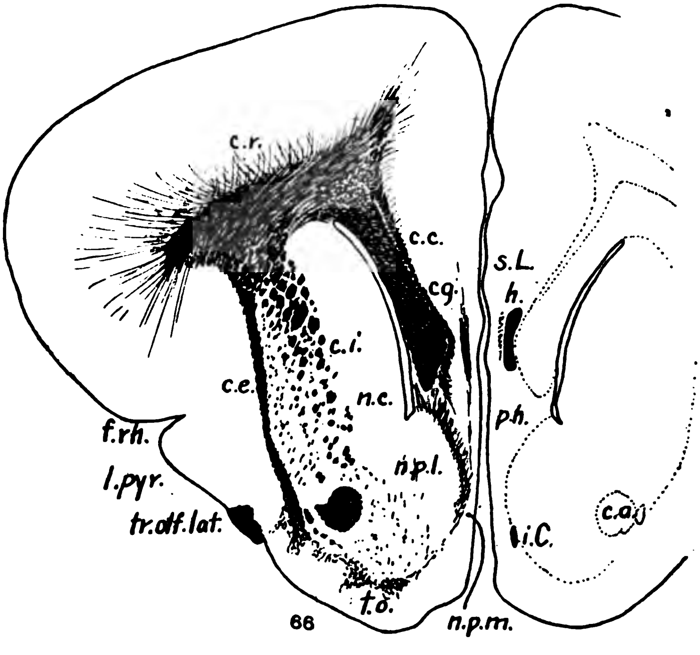
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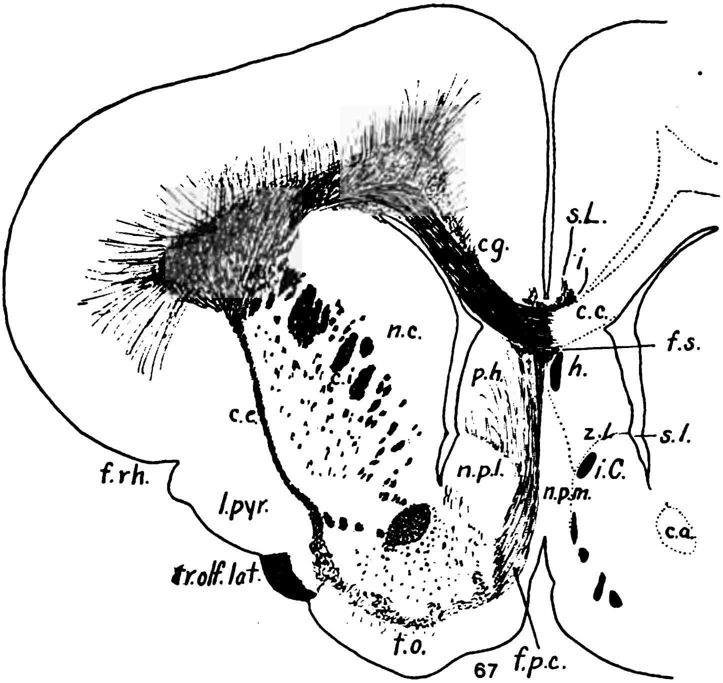
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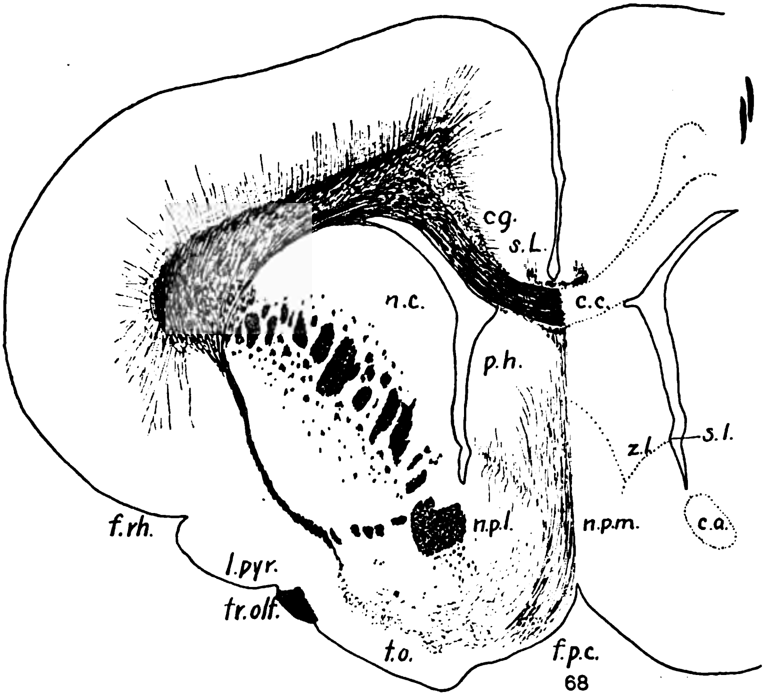
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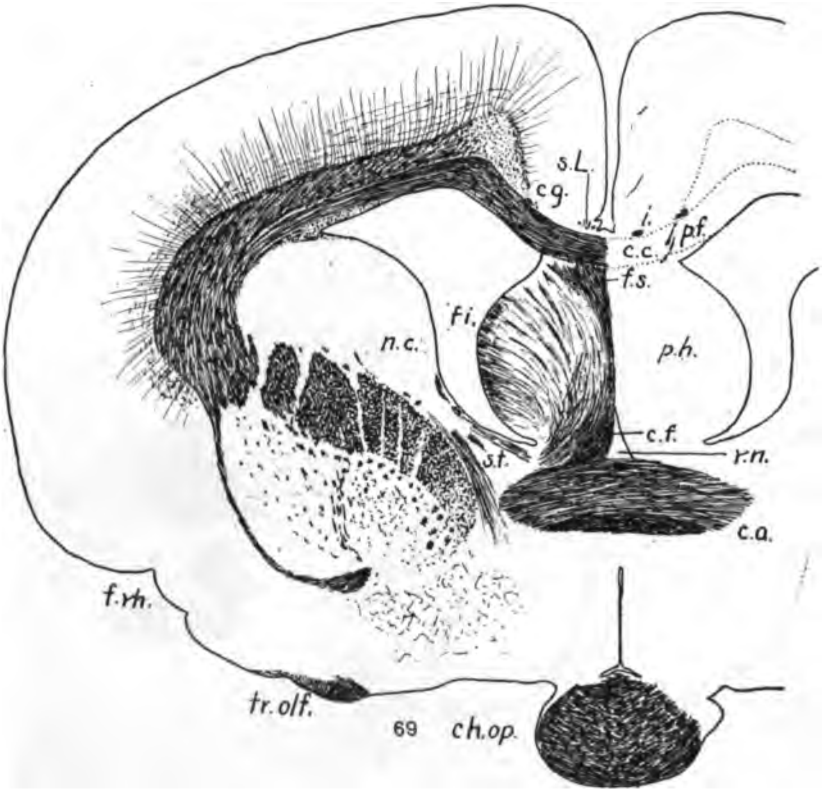


Fig. 67 Section caudal to the genu. A small band of hippocampal cortex is seen beneath the genu as in the rat. The sharp separation of the hippocampal primordium from the parolfactory area is more clear in the rabbit than in most mammals.

Fig. 68 Section a short distance in front of the anterior commissure. Note how the precommissural fibers in part contribute to the fornix superior and in part enter the hippocampal primordium.

Fig. 69 Section at the level of the neuroporic recess. The fornix columns contribute to the fornix superior. The perforating fibers appear to enter the cingulum. It is possible that they go to the hippocampus farther back.

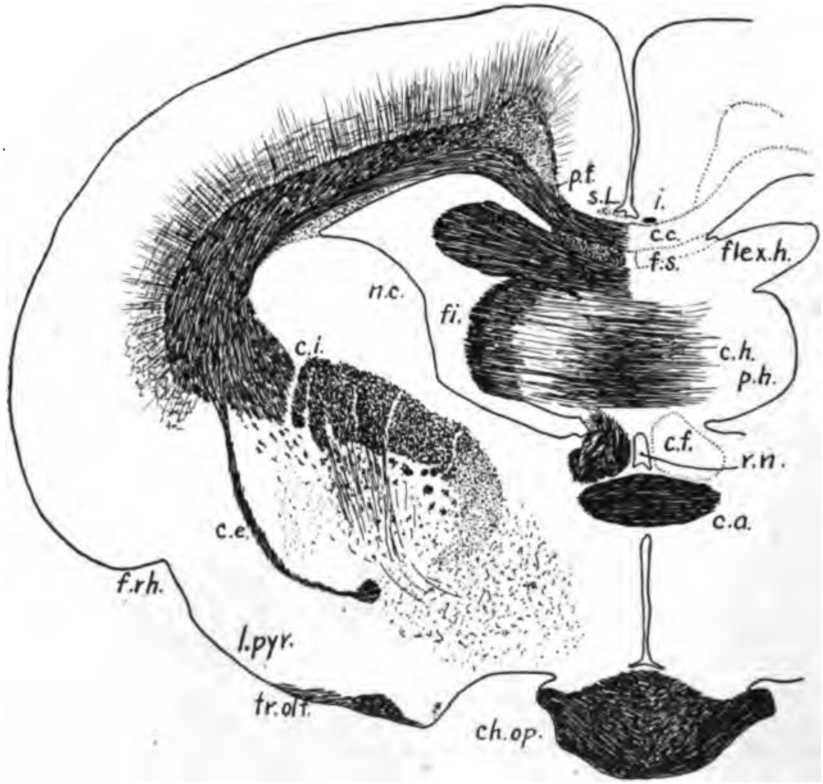
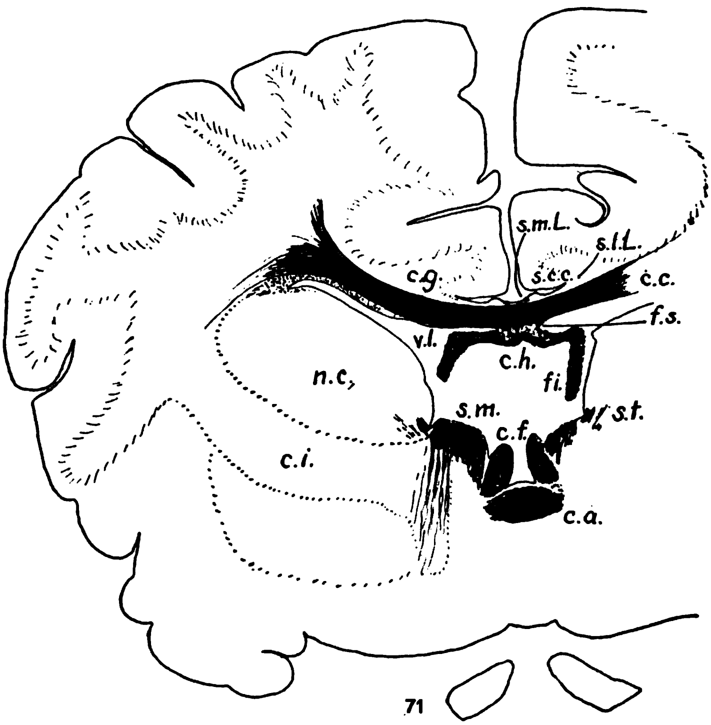


Fig. 70 Section just in front of the foramen. Description in the text.



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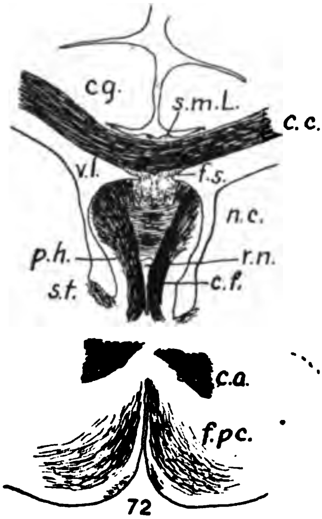
Figs. 71 to 75 Bear (Ursus americanus). Five transverse sections from a Weigert series. Magn. 2 diam.

Fig. 71 Section through the interventricular foramen. The section cuts the caudal border of the anterior commissure. The base of the neuroporic recess is seen between the fornix columns. It extends forward through the whole thickness of the anterior commissure (see figs. 72 and 90). Note the prominent striae Lancisii. The fimbria is free from gray matter but a few cells accompany the fornix superior.



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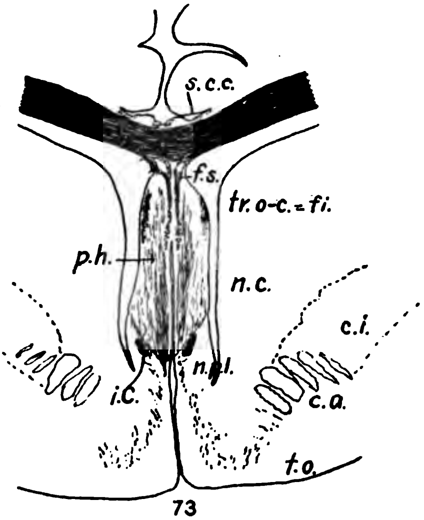

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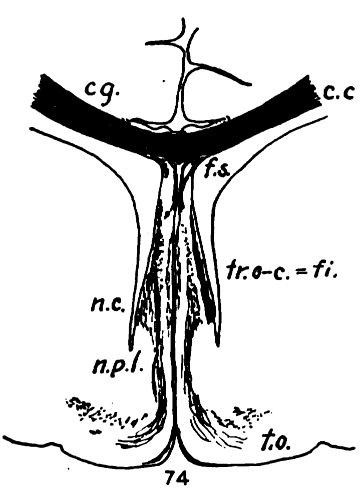

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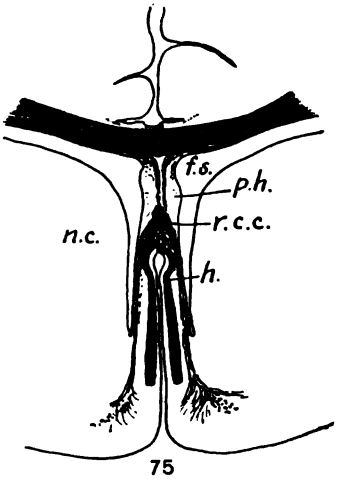

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Fig. 72 Section through the hippocampal commissure, fornix columns and neuroporic recess.

Fig. 73 Section 2.15 mm. rostral to the last. The zona limitans is sharply marked by the precommissural fibers as in the opossum and rabbit. Islands of Calleja come up to this level as in the other forms.

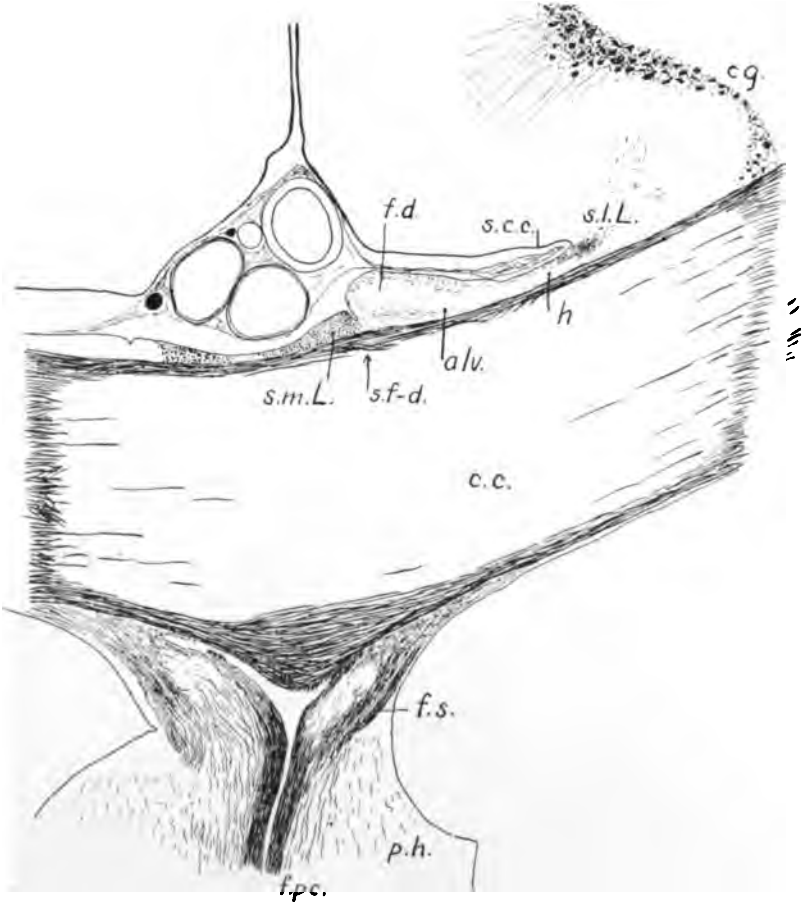

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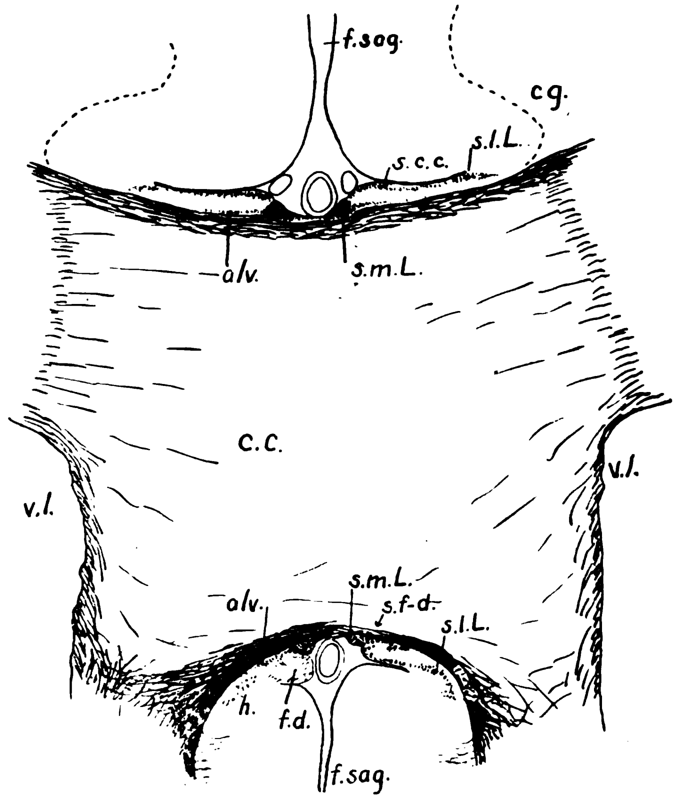
Fig. 74 Section 1.1 mm. rostral to the last. Note that in the bear the primordium hippocampi has become almost thin enough to be called a septum pellucidum.

Fig. 75 Section behind the genu and cutting the rostrum beneath. Note that here the indusium coming down around the genu as in all forms is continued down from the rostrum toward the olfactory peduncle. It is diagrammatically represented in black. This is essentially the condition of the hippocampus in the embryos of higher mammals and man.

Fig. 76 Bear, a part of the section shown in figure 73, drawn at a higher magnification. Description in the text.



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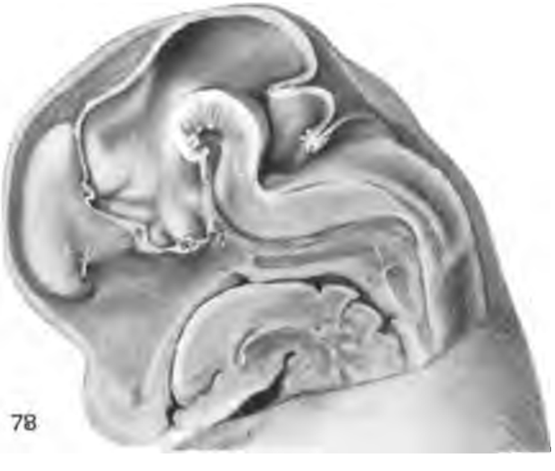

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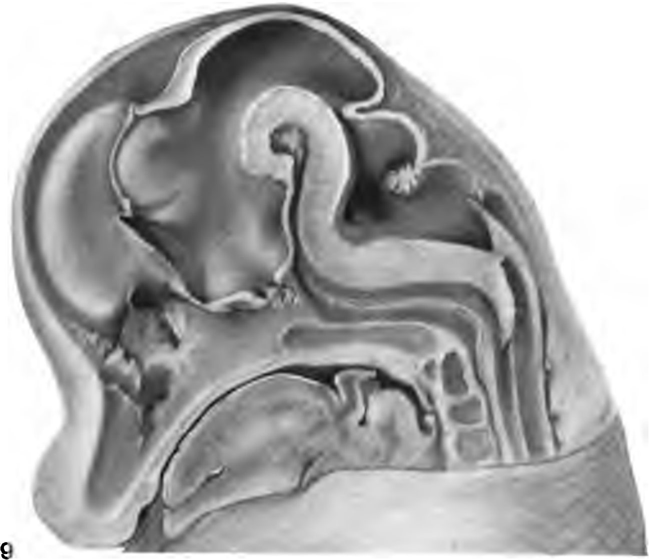
Fig. 77 Bear, section through the genu showing that the indusium as it curves beneath the genu develops a complete, though small, hippocampal formation with all the typical parts present. Compare figures 29, 43 and 58.

Fig. 78 Pig embryo, 23 mm. Medial surface of the right half of the head. The lamina terminalis contains the anterior commissure. The lamina supraneuroporica is only slightly thickened. Between it and the velum transversum is the paraphysal arch, the angulus terminalis of His.



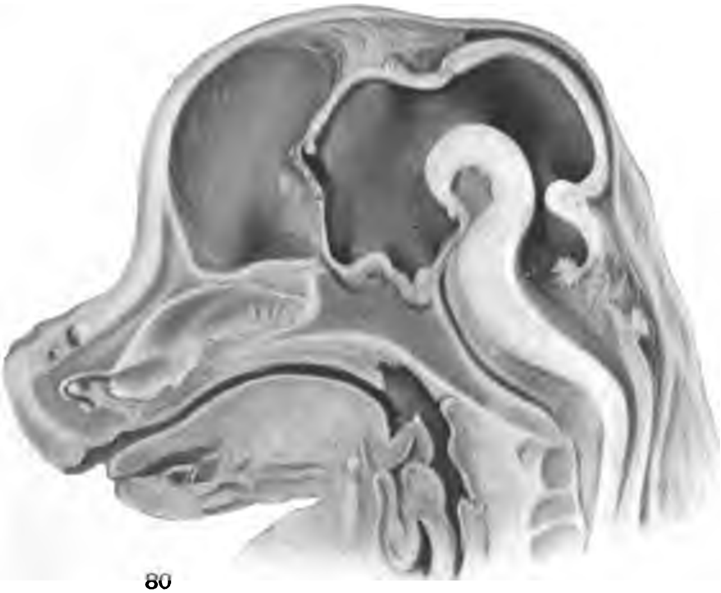


Fig. 79 Pig embryo, 28 mm. The lamina supraneuroporica is raised to a more vertical position by the growth of the hemisphere and the thalamus is relatively crowded. There is still no commissure visible in the lamina supraneuroporica but it is somewhat thicker than in the 23 mm. stage.

Fig. 80 Pig embryo, 40 mm. The lamina supraneuroporica is still more elevated and the paraphysal arch is crowded into a deep narrow sac, in front of the developing choroid plexus. Now the anterior pallial commissure is evident as a fibrous mass occupying the lamina supraneuroporica. There is up to this time no secondary fusion nor extensive thickening in the region of the commissures.

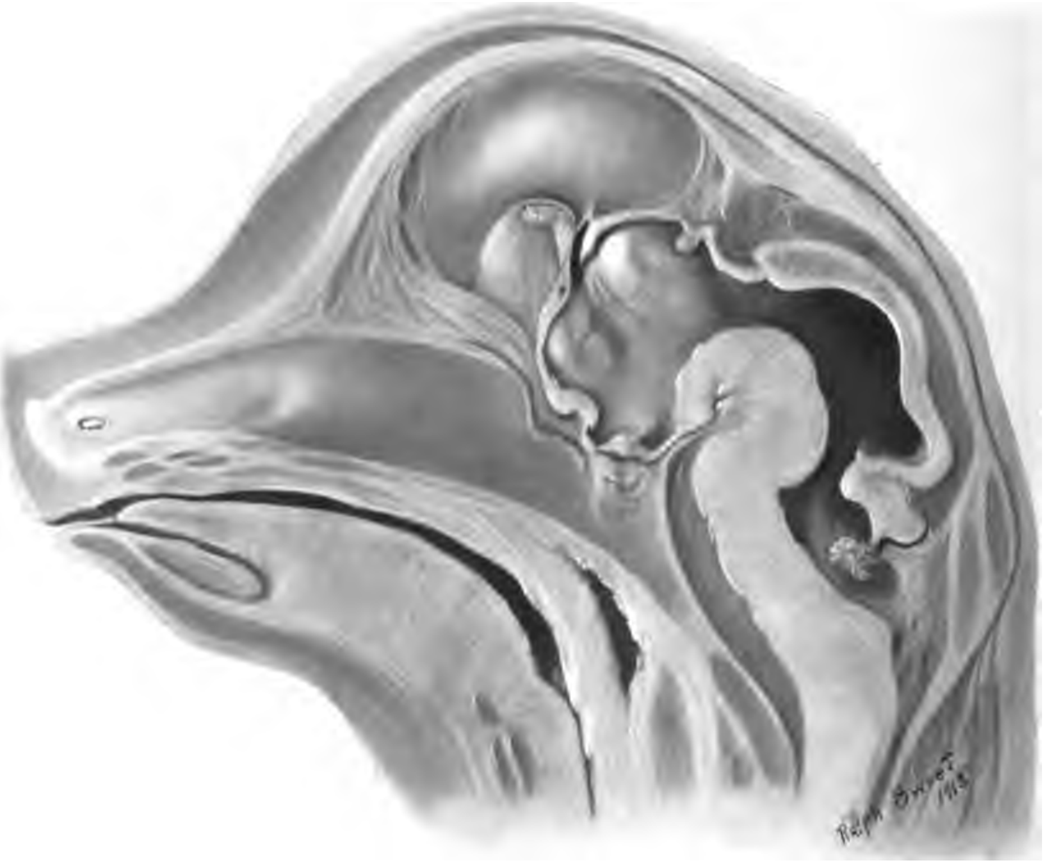
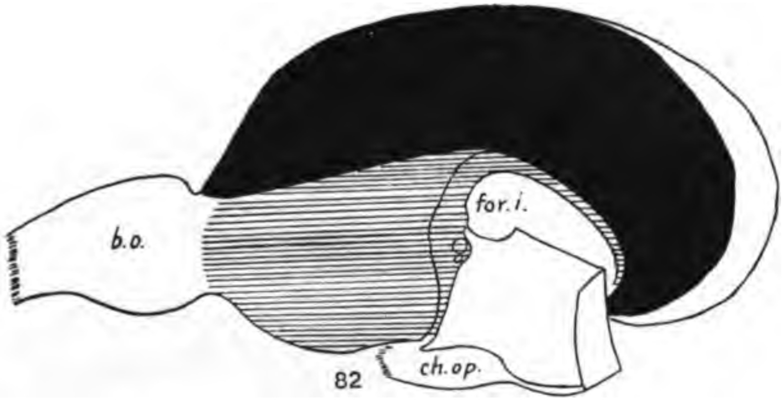


Fig. 81 Pig embryo, 50 mm. Rapid development of the anterior pallial commissure has produced a condition very similar to that of the adult bat. The commissure still occupies the lamina supraneuroporica but also extends forward from its dorsal border to form a crescent. Above the crescent is seen the indusium in a condition very much like that of the hippocampal formation in the opossum or bat. I can find no indication of secondary fusion. The rostral limb of the commissure is formed simply by the invasion and stretching of the dorsal border of the lamina supraneuroporica by additional corpus callosum fibers.



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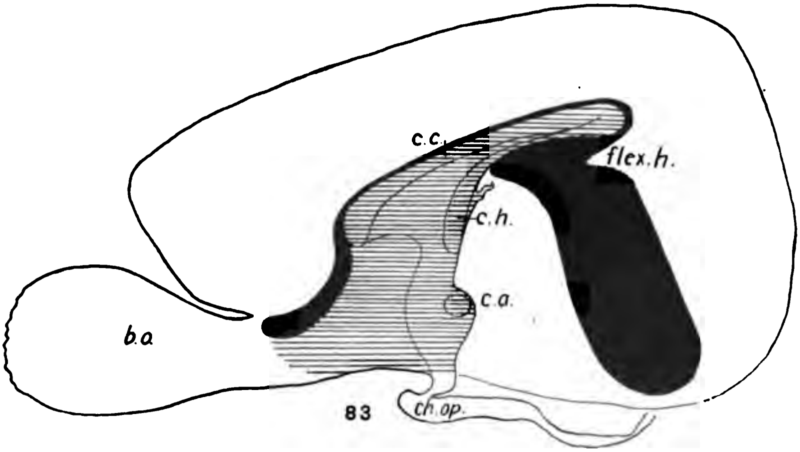

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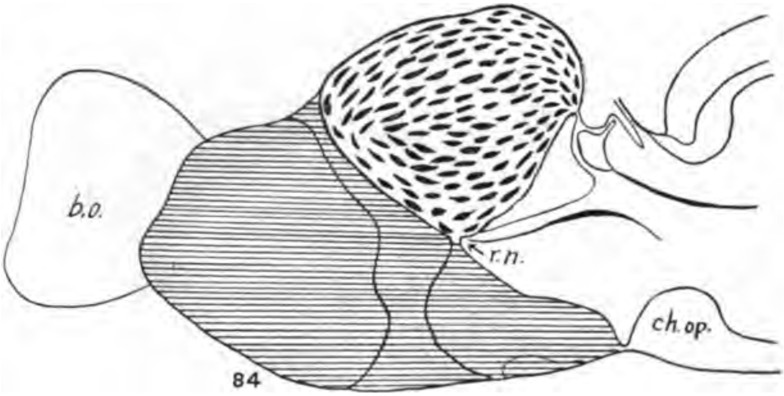
Figs. 82 to 90 Diagrams to illustrate the relations of the hippocampus, hippocampal primordium and paraterminal body. The medial surface of the right hemisphere is drawn in each case. The paraterminal body is shaded with horizontal lines, the hippocampal primordium is distinguished by means of spindle-shaped spots or flecks and the hippocampus is in solid black. The commissures are merely outlined with pen lines. All the diagrams except those of the bat and the bear are drawn from dissections.

Fig. 82 Diagram of the brain of a reptile to illustrate the relations of hippocampus and paraterminal body as defined by Elliot Smith. The outline is taken from the turtle's brain. Herrick recognizes that the narrow ridge over the foramen in the turtle is primordium hippocampi but describes a much larger supraforaminal portion of the paraterminal body in lizards.

Fig. 83 Diagram of the brain of the rat similar to the last. The whole commissural system is supposed to be imbedded in the paraterminal body.



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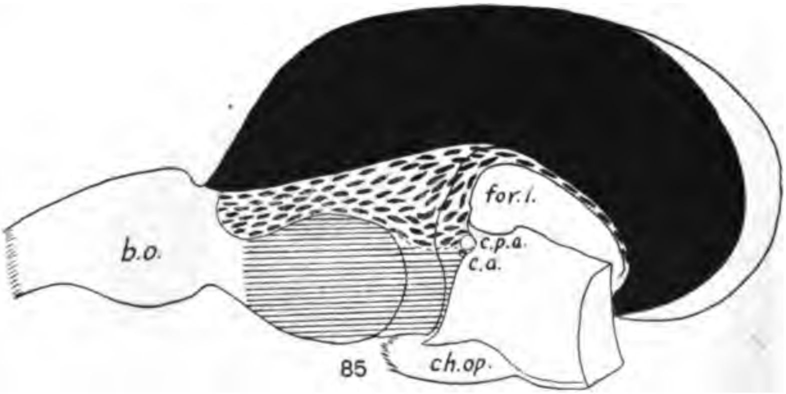

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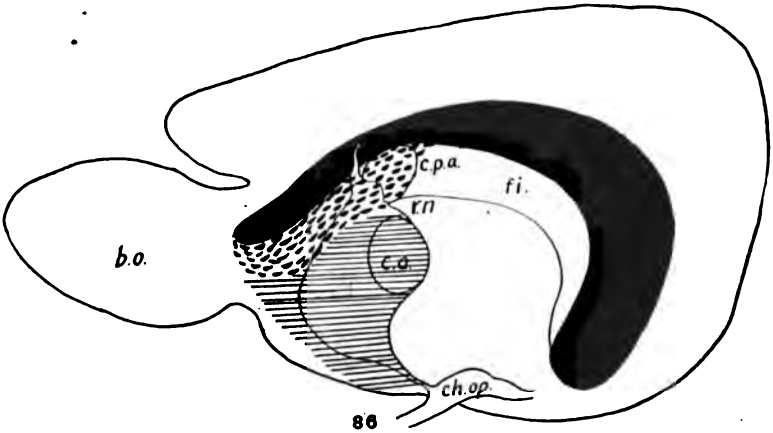
Fig. 84 Diagram of the forebrain of Scyllium to illustrate the author's conception of the relations. The hippocampal primordium is separated from the paraterminal body by the neuroporic recess. This primordium gives rise to two structures in higher vertebrates: hippocampal formation and a residual body less highly organized which is represented in mammals by the septum pellucidum. This residual structure is represented in the following diagrams by the flecked or spotted area.

Fig. 85 Diagram of the brain of the turtle to illustrate the view set forth in this paper. Not only in the turtle but in lizards and other reptiles the body which runs along over the foramen, between it and the hippocampal cortex, is hippocampal primordium.



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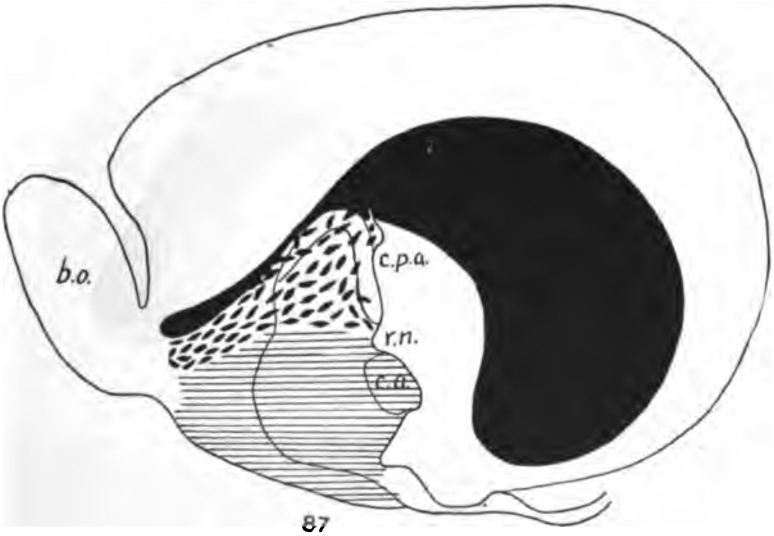

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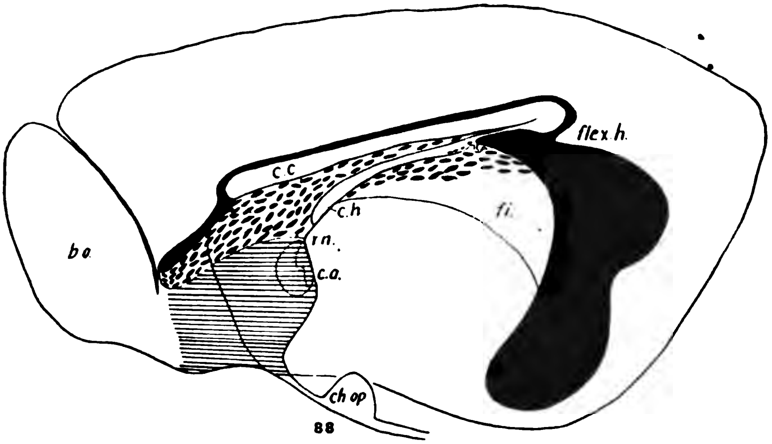
Fig. 86 Diagram of the brain of the opossum. This type of brain has been made familiar by Elliot Smith's work. The reasons for assigning a portion of Professor Smith's paraterminal body to pallial area are set forth in the text.

Fig. 87 Diagram of the brain of the bat. The commissures are poorly developed and there is nearly as close connection between hippocampus and its primordium as in the opossum.



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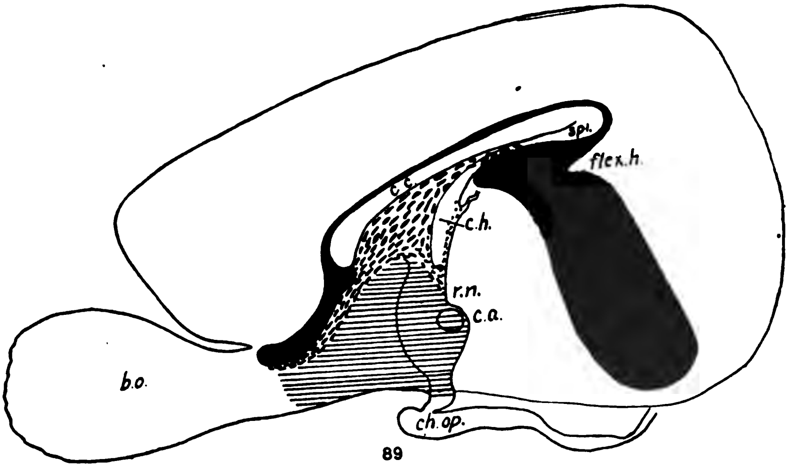

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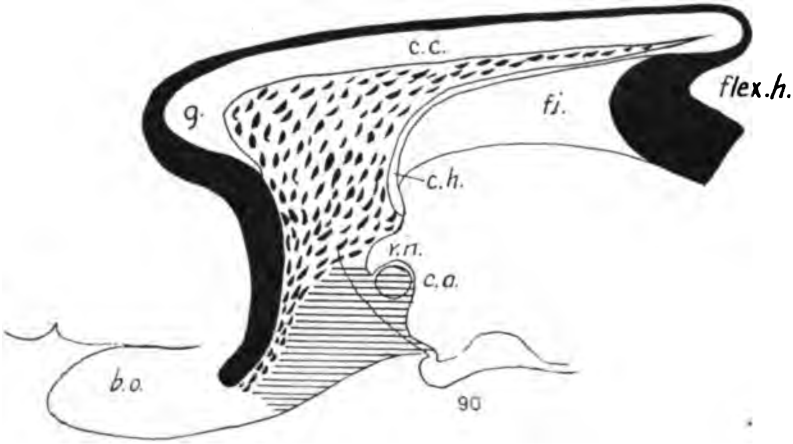
Fig. 88 Diagram of the brain of the mole. Although there is a large corpus callosum and a splenium is well formed, there is an unusually large primordium reaching almost the entire length of the corpus callosum and connecting with the hippocampal flexure. The boundary of the paraterminal body as it appears in the ventricular surface is represented here. Reference to figure 55 will show that the medial parolfactory nucleus rises much higher on the outer surface. The diagram of the rat brain following is drawn with reference to the boundary on the outer surface.

Fig. 89 Diagram of the brain of the rat. The gradual merging of the indusium beneath the genu with the primordium is represented by tooth-like projections. The paraterminal body rises higher on the medial surface than it does next the ventricle. On the ventricular surface the boundary line runs almost straight forward from the neuroporic recess.



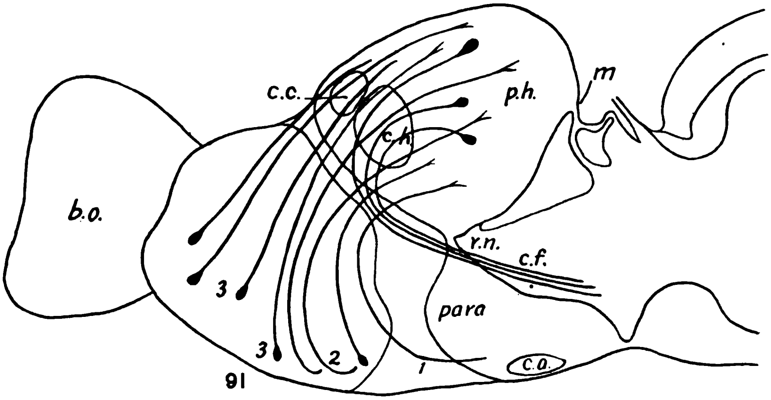
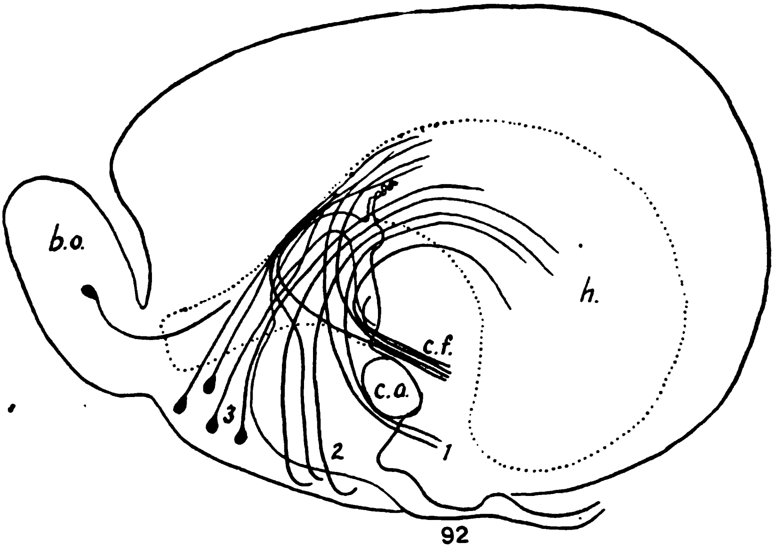


Fig. 90 Diagram of the septal region in the brain of the bear, based on serial transverse sections. The hippocampal commissure is a very thin band. The gray matter in the caudal part of the septum is reduced to a very slender strand accompanying the fibers of the fornix superior.

Figs. 91, 92, 93 Diagrams of the brains of Scyllium, the bat and the rat to illustrate the relations of the fibers of the fornix system to the pallial commissures. See section on this subject in the text. The arabic numerals *1*, *2*, *3*, have the same significance as in figure 38.



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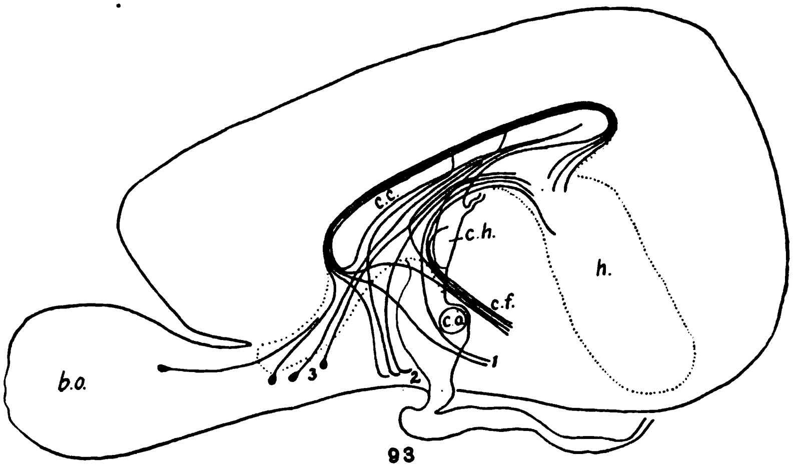

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44,960

CONTRIBUTIONS
FROM THE
DEPARTMENT OF ANATOMY

VOLUME 3

1914-1915

MINNEAPOLIS
Published by the University of Minnesota
1916

MAY 30 1916

Transferred to
Museum of Comparative Zoology
20 Jun 1917

CONTENTS

The following contributions have been published during the years 1914-1915:

Baumgartner, E. A. 1. The development of the hypophysis in Squalus acanthias. *Journal of Morphology*, 26 (1915): 391-446, 43 figures.

2. The development of the gall bladder and bile ducts in Amblystoma. (Abstract.) *Anatomical Record*, 8 (1914).

Hoskins, E. R. 1. On the vascularization of the spinal cord of the pig. *Anatomical Record*, 8 (1914): 371-391, 5 figures.

2. Persistent arteriae brachii superficialis, antibrachii superficialis et mediana. *Anatomical Record*, 8 (1914): 421-422, 1 figure.

3. On the development of the digitiform gland in Squalus acanthias. (Abstract.) *Anatomical Record*, 9 (1915).

Jackson, C. M. 1. *Morris' Human Anatomy;* Introduction and sections on Morphogenesis and Digestive System. 5th ed. Philadelphia, 1914.

2. Effect of acute and chronic inanition upon the relative weights of the various organs and systems of adult albino rats. (Abstract.) *Anatomical Record*, 9 (1915).

3. Changes in young albino rats held at constant body-weight by underfeeding for various periods. (Abstract.) *Anatomical Record*, 9 (1915).

4. Effects of acute and chronic inanition upon the relative weights of the various organs and systems of adult albino rats. *American Journal of Anatomy*, 18 (1915): 75-116, 2 figures.

5. Changes in the relative weights of the various parts, systems, and organs of young albino rats held at constant body-weight by under-feeding for various periods. *Journal of Experimental Zoology*, 19 (1915): 99-156, 4 figures.

6. Obstacles to research. *Science*, N.S., 42: 819-823, (Dec. 10, 1915).

Johnston, J. B. 1. The nervus terminalis in man and mammals. *Anatomical Record*, 8 (1914): 185-198, 9 figures.

2. A tractus olfacto-tegmentalis in the human fetal brain. *Journal of Comparative Neurology*, 25 (1915): 283-290, 9 figures.

3. The cell masses in the forebrain of the turtle, Cistudo carolina. *Journal of Comparative Neurology*, 25 (1915): 393-468, 60 figures.

Lee, Thomas G. 1. On the implantation and placentation in the sciuroid rodents. (Abstract.) *Anatomical Record*, 9 (1915).

2. On the relationship of the endocardium to entoderm in Citellus. (Abstract.) *Anatomical Record*, 9 (1915).

3. The passage of the ovum through the uterine epithelium in Geomys bursarius, with demonstration of wax reconstructions. (Abstract.) *Anatomical Record*, 8 (1914).

4. An improved electric microscope lamp, with demonstration. (Abstract.) *Anatomical Record*, 8 (1914).

Myers, J. A. 1. Studies on the syrinx of Gallus domesticus. (Abstract.) *Anatomical Record*, 9 (1915).

Scammon, Richard E. 1. The development of the pancreas in selachians. (Abstract.) *Anatomical Record*, 8 (1914).

2. The technique of Weber's method of reconstruction. (Abstract.) *Anatomical Record*, 9 (1915).

3. On Weber's method of reconstruction and its application to curved surfaces. *Anatomical Record*, 9 (1915): 247-258, 5 figures.

4. The histogenesis of the selachian liver. *American Journal of Anatomy*, 17 (1915): 245-315, 45 figures, 7 plates.

Reprinted from JOURNAL OF MORPHOLOGY, Vol. 26, No. 3
September, 1915

THE DEVELOPMENT OF THE HYPOPHYSIS IN SQUALUS ACANTHIAS

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FORTY-THREE FIGURES

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INTRODUCTION

Although considerable work has been done on the hypophysis of elasmobranchs, many points still remain concerning which there has been much discussion and which evidently require further investigation. It was thought that an intensive study of the history of this organ in one form might be of value.

The embryos used are from the collection of Dr. R. E. Scammon and from the large series which form a part of the embryological collection of the Harvard Medical School.[2] For the pup and adult stages special sections and dissections were made. The difficulties in making a dissection of the hypophysis in selachians have been noted by other investigators. In the present work, it was possible only after numerous attempts to obtain a dissection showing the ventral lobes connected with the rest of the hypophysis. Graphic and wax reconstructions of different embryos and parts of the adult Squalus were made.

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[1] This work was begun while at the University of Minnesota and completed at Washington University Medical School.

LITERATURE

1. *Embryology*

The literature on the development of the elosmobranch hypophysis may properly be divided into two categories, one on the embryology and the other on the adult anatomy and histology of this organ. Although the earlier work on the hypophysis in elasmobranchs concerned its adult anatomy, probably investigators have occupied themselves more with its development. Some, of course, have given attention to both the adult anatomy and the development in the same paper or in a series of articles.

Müller ('71) confirmed Rathke's earlier observations that the hypophysis is developed from the mouth. In Acanthias vulgaris and other selachians he found that it was composed of a principal posterior part and a secondary anterior part. He described the position and gave measurements of the size of the hypophysis and of the thickness of different parts of the wall in an Acanthias embryo 30 mm. long. In a 10 cm. embryo of Mustelus he described the tubular masses forming the hypophysis.

Balfour ('74) briefly stated that the hypophysis is an outpouching from the mouth. In 1878 he described the hypophysis as being somewhat constricted from the mouth by the close of

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[2] Through the courtesy of the late Dr. Charles S. Minot I was able to make use of the extensive Harvard Embryological Collection and had the privileges of his laboratory during the summer of 1914. I also wish to thank Dr. J. S. Kingsley and others of the South Harpswell Laboratory for the courtesies of that laboratory for a part of the summer.

'Stage K.' Soon after this its terminal part dilates and the hypophysis is completely constricted from the mouth.

Reichert ('77) in a description of some cleared embryos of Acanthias remarked on the position of the hypophysis and its relation to the notochord. He opposed the theory of the ectodermal origin of the hypophysis in Acanthias.

Rabl-Rückhard ('80) in his work on the relations of the notochord, confirmed Müller's observations concerning the hypophysis. He stated that there is a ventral secondary outpouching from the hypophysis in a 60 mm. Acanthias. In an embryo corresponding to Stage K of Balfour he has perhaps mistaken the connection between the premandibular somites for the hypophysis. This seems probable from the position as well as from the fact that he found no connection between this structure and the mouth at this time.

In 1888 Edinger published a paper on the comparative anatomy of the forebrain. He stated that in Torpedo the hypophysis is at first a simple outpouching of the mouth which later develops secondary outpouchings.

Sedgwick ('92) in his work on Scyllium and Raia made the statement that the first rudiment of the mouth extends into the pituitary body.

Von Kupffer ('94) reviewed the literature on the development of the hypophysis and gave some results of his own work. He thought that the hypophysis is not derived entirely from ectoderm, and his observations on other vertebrates supported his view that the hypophysis is partly of entodermal origin.

Hoffmann ('96) described the early stages in Acanthias development. He stated that the hypophysis develops entirely from ectoderm. He found the first definite outpouching anterior to the buccopharyngeal membrane in 13 to 14 mm. embryos. He also described a yellowish pigment in the buccopharyngeal membrane which could still be seen in the stalk of 25 mm. embryos.

Haller ('96) gave an account of the development of the hypophysis in Mustelus. He described the position of the organ in a 22 mm. embryo and called attention to the low epithelium found at the opening of the hypophysis into the mouth. The connection with the mouth is soon lost and in a 90 mm. stage the whole structure has come into closer relation with the brain floor and vascular sac, and has grown forward. The roof of the hypophysis has become thickened, as have the anterior and posterior ends. From the floor of the caudal end two lateral outpouchings have developed which later form the inferior sacs. In a 20 cm. Mustelus all parts of the hypophysis are quite well developed. A vascular layer separates the roof from the brain floor. The roof of the inferior sacs becomes thinner. Haller also described the interhypophyseal canal joining the inferior sacs to the superior part. He figured glandular outgrowths extending forward from it. From the floor of the anterior sac are several prolongations extending anteriorly while from the roof are many small outpouchings. The extreme anterior end shows many mitotic figures and is broken up into a network by many capillaries. The floor at the cephalic end of the anterior lobe is very thin—this, Haller stated, is where the hypophysis is constricted from the mouth and here the epithelium has remained of a low cuboidal type. The head of the organ is composed of many closely-crowded tubules. These develop first as solid evaginations, then the nuclei separate and rearrange themselves, later a cleft appears in the protoplasm, which cleft ultimately connects with the main lumen.

Chiarugi ('98) briefly considered the hypophysis in a paper on the description of a prehypophyseal body and the hypophyseal area in Torpedo ocellata. He described a connection between the premandibular somites and the hypophysis and figured a median sagittal section of a 15 mm. embryo which showed a constriction of the hypophysis from the mouth.

Nishikawa ('99) noted that the hypophysis is present as a simple outpouching in 32 mm. Chlamydoselachus embryos. He made a series of drawings of transverse sections which show its position at this stage.

Sewertzoff ('99) in studying the development of the selachian skull took up the interrelation of development of the skull and brain. The trabeculae and parachordal plates in Acanthias and Pristiurus are almost at right angles to each other in early stages (figs. 25 and 29). Later a bend in the medulla and a corresponding one in the parachordal plate changes the relation of the two cartilages. Also the forebrain has shifted from a position ventral to the medulla to a more dorsal and rostral one (fig. 1). Sewertzoff showed the change in position of the hypophysis occurring with the change in position of the skull and brain (fig. 23).

Rossi ('02) described the development of two lateral lobes in Torpedo. These, he stated, develop very early from the lateral walls of the evagination forming the hypophysis. He homologized the different portions of the hypophysis as he found them with those described by Haller, but described two special lateral lobes which he stated have no homologous parts in Mustelus according to Haller's description.

Gentes ('06, '07) found a close relationship between the vascular sac and the underlying hypophysis. In several short reports ('08) he described the lateral lobes and the development of the inferior lobes of the hypophysis. These two parts, he stated, form a ventral pituitary body. In a longer paper this author ('08) gave the results of his studies on the development and evolution of the hypophysis in Torpedo. He described two main parts, the superior and inferior sacs. The superior sac is further divided into posterior and anterior parts. The hypophysis begins as an outpouching which in 45 mm. embryos shows a beginning of its division into the two main sacs. From the posterior part of the superior sac many cords grow dorsalward, these later becoming tubular. Gentes homologized the superior lobe with the anterior lobe of mammals. The lateral lobes he believed persist through life.

Ziegler ('08) in a series of figures of an embryo of Chlamydoselachus, corresponding to Balfour's Stage L–M, showed the hypophysis as an upward anterior-extending evagination from the mouth. The connection with the mouth is still a wide canal.

Johnston ('09) described the hypophysis in Acanthias as consisting of a short anterior portion which grows toward the optic chiasma, and a longer posterior lobe directed toward the vascular sac.

Scammon ('11) in the normal plate series has figured the hypophysis in several of the younger stages. He described a distinct outpouching in 7.5 mm. embryos. In 18 mm. embryos a constriction of the anterior part of the hypophysis from the mouth has begun. In 24 mm. embryos shallow furrows separate two lateral portions from a median portion in the posterior part. A slight lateral constriction separates the anterior and posterior lobes.

In 1912 Sterzi described the development of the hypophysis in Acanthias. He noted that the front wall of the outpouching becomes dorsal in later embryos. A rostral lobe develops which is in part anterior to the stalk connecting the hypophysis to the mouth. Two lateral outpouchings arise which later form the endocranial portion. The dorsal lobe develops at the superior end of the early outpouching. An early differentiation takes place in the cells of the dorsal lobe. Buds grow out from this thickened wall and form epithelial cords between which are blood vessels and nerve fibers. Differences in the affinity for stains distinguish the rostral (and endocranial) lobes from the superior which is the chromophobic lobe in adults.

2. *Anatomy and histology*

Von Michlucho-Maclay ('68) described in Acanthias and in Scymnus a persistent connection between the mouth and the hypophysis. In his later work ('70) this investigator failed to find such a connection and believed his former observations to be incorrect.

Müller ('71) found in later embryos and adults of Acanthias that the cells forming the glandular part are columnar while those toward the periphery of the cords and tubules are spindle-shaped, with a finely granular cytoplasm. He also noted the large capillaries and the connective tissue between the tubules.

Viault ('76) briefly described the hypophysis in Raia. The hypophysis is surrounded by a connective tissue sheath which sends strands into the organ carrying large capillaries between the convoluted tubules. These tubules are 0.015 to 0.007 mm.

in diameter and usually have a small lumen. They are composed of cylindrical cells. The tongue-like anterior end, he stated, was of similar structure. He was of the opinion that the glandular portion arose from the mouth.

Rohon ('79) described an hypophysis composed of tubules in selachians. It is a triangular shaped organ with a long tongue-like projection which extends forward almost to the optic chiasma. He found no cavity within it. In Acanthias and Mustelus the hypophysis is larger than in Torpedo and Scyllium.

Sanders ('86) studied the central nervous system of Scyllium and Acanthias. He stated that the hypophysis is attached to the infundibulum by a glandular tube which lies between the inferior lobes. In a drawing of the brain of Acanthias (lateral view) he figured the hypophysis as lying caudal to the inferior lobes of the mesencephalon.

In 1892 Edinger described the mid-brain region. In Torpedo and Scyllium he found the hypophysis composed of tubules and cords of epithelium, among which are many blood vessels. He noted nerve fibers extending from the infundibular region ventralward into the hypophysis.

Haller ('96) described in detail the structure of the hypophysis of a 20 cm. Mustelus. He stated that in the adult the glands of the head (superior) portion are tubular. Secretion and cell detritus are found in the lumina. He also described an opening in the base of the anterior lobe. This is found in the thin portion of the floor in the position of the original connection with the mouth, and connects the cavity of the hypophysis with the subdural space.

Sterzi ('04) described the hypophysis in several selachians. He found the anterior part flattened dorso-ventrally, extending almost to the optic chiasma and containing a large cavity. The posterior part is separated from the remainder by connective tissue. A slender canal connects this to the anterior portion. The superior part is formed of anastomosing cords interlacing with sinusoids. The cords are formed of a peripheral layer of columnar cells and an inner mass of polyhedral ones. The nuclei of the outer zone are rich in chromatin. The polyhedral cells contain small spherical nuclei. The cytoplasm is granular and stains deeply.

Pettit ('06) made a study of the hypophysis in Centroscymnus. He mentioned posterior, median and anterior parts, all communicating. He found ramifying cords or tubules surrounded by sinusoids.

Burckhardt ('07, '11) in his work on the central nervous system of selachians described, incidentally, the hypophysis in Scymnus. He recognized a terminal, a median and a posterior lobe. Apparently he included under his division of median lobe, the caudal end of the anterior lobe of Sterzi, or it may be that the hypophysis in Scymnus is quite different from that found in other forms.

Joris ('08, '09) described the dorsal lobe in Spinax and Mustelus as formed in part from the hypophyseal evagination and in part from the infundibulum. The cell cords arise from the hypophysis while neuroglia and nerve fibres from the infundibular region grow in between these.

In 1909 Sterzi in his comprehensive work on the central nervous system of selachians, gave a clear description of the hypophysis. According to this description the hypophysis is composed of perimeningeal and endocranial parts. The perimeningeal portion is further subdivided into a dorsal and a rostral lobe. The dorsal lobe has many columns from its dorsal surface. Among these are numerous capillaries. The rostral lobe is a long flattened part extending almost to the optic chiasma. The endocranial part is composed of two sacs connected medially to one canal which leads to the anterior or rostral lobe. The cords and tubules of the dorsal lobe anastomose forming a network in which is a rich vascular supply. These are primarily attached by means of connective tissue to the base of the brain or vascular sac. The structure of these cords, Sterzi had described before ('04). The dorsal lobe, because it stained so lightly he termed the chromophobic portion. The rostral lobe has tubules extending from its ventral walls. The ventral wall has a distinct median ventral furrow and the tubules of either side remain separated. The distal ends of the tubules are solid and by means of branches anastomose with the cords of the other tubules. The arrangement of the cells here is the same as that in the dorsal lobe. This portion stains more deeply, the capillaries are smaller and less numerous. The endocranial portion in embryos is a sac with folded walls. In the adult, many tubules and cords have developed. In the main this resembles the rostral portion of the perimeningeal part.

Tilney ('11) in his studies on the comparative histology of the hypophysis, stated that in Acanthias there is a distal epithelial portion made up of parallel cell columns. The cells are deeply acidophilic with only a few faintly-staining acidophilic ones present. In the juxta-neural part the cells are larger, usually irregularly disposed, but forming some acini. However, they take the basic stains very markedly. He found this portion less vascular than the distal part. Tilney has homologized the different portions of the hypophysis in the various vertebrate groups. The distal epithelial portion of selachians corresponds to the intermediate lobe of mammals.

In 1913 Stendell made a comparative study of the hypophysis. He described intermediate and main lobes in Heptanchus. In the main lobe he described the peripheral cells of the tubules as of an acidophilic nature, while those toward the center are basophilic or neutral. Stendell has homologized the parts found in the hypophysis of selachians with those in the higher forms of vertebrates. His homology agrees with that of Tilney, i.e., the intermediate lobe of Heptanchus is homologous with the same portion in the higher vertebrates, being in Heptanchus very large and becoming smaller in the higher forms. The ventral sac of the main lobe is not found in higher forms.

There have been many terms used in the descriptions of the different parts of the elasmobranch hypophysis. A comparison with figure 1, which is a drawing of a model of the hypophysis of a pup, and a table of the terms employed may serve to explain the terms used in reviewing the literature.

TABLE 1

Terms used by the various investigators for the different parts of the elasmobranch hypophysis

OBSERVER	MATERIAL	REGIONS OF THE HYPOPHYSIS			
		Anterior	Inferior	Superior	Special portions
Müller ('71).......	Scymnus, Acanthias	Anterior part (secondary)	Posterior (main part)		
Haller ('96)........	Mustelus	Superior sac	Inferior sac	Head	
Rossi ('02)........	Torpedo	Median lobe; antero-median diverticulum	Antero-lateral diverticulum	Terminal lobe	Lateral lobes
Sterzi ('04)........	Mustelus, Acanthias	Anterior portion	Posterior portion	Superior portion	
Pettit ('06)........	Centro-scymnus	Anterior	Median	Posterior	
Burckhardt ('07)..	Scymnus	Terminal lobe	Posterior lobe	Median lobe	
Gentes ('07)........	Torpedo	Superior sac; anterior two-thirds	Inferior sac	Superior sac; posterior one-third	
Joris ('08)..........	Mustelus		Posterior	Dorsal	
Sterzi ('09)........	Acanthias	Perimeningeal portion; rostral lobe	Endocranial portion	Perimeningeal portion; dorsal lobe	
Tilney ('11).......	Acanthias	Juxta-neural part		Distal part	
Stendell ('13)......	Heptanchus	Main lobe	Ventral sac of main lobe	Intermediate lobe	
Sterzi, Gentes, et al...............		Chromophilic portion		Chromophobic portion	

MORPHOLOGY AND MORPHOGENESIS

1. *Description of the hypophysis in the pup stage*

A description of the hypophysis in the pup stage may make clear the different parts of this organ. The formation of tubules has begun and the main portions or lobes are well formed at this time.

The anterior lobe is a tongue-like process with two somewhat wider extremities; these are connected by a more slender middle part which is less than one-third of the entire length. There is a deep sulcus in the median ventral wall which extends from about the middle of the posterior extremity to near the end of the anterior extremity (fig. 1). Several other more or less regular furrows occur in the ventral surface of the anterior extremity. Two lateral constrictions separate the middle portion from the some-

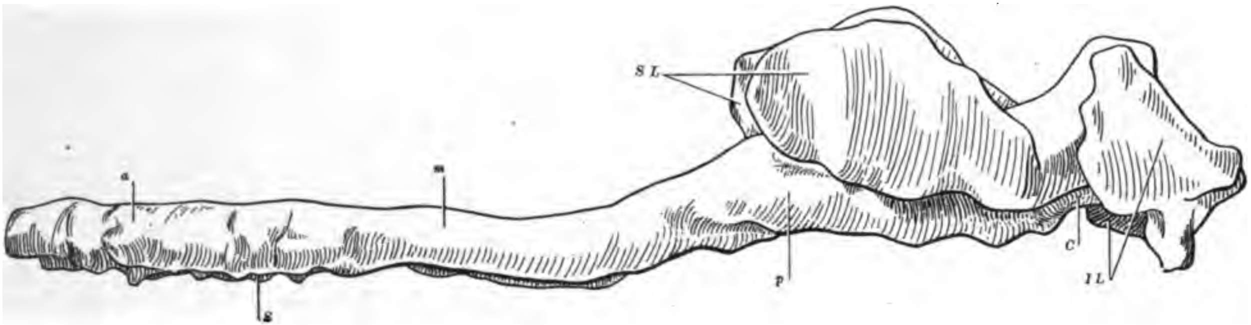


Fig. 1 Left lateral view of a reconstruction of the hypophysis of an Acanthias pup. × 50. *a*, *p*, anterior and posterior extremities of the anterior lobe; *C*, interhypophyseal canal; *IL*, inferior lobe; *m*, mid part of anterior lobe; *S*, median ventral sulcus; *SL*, superior lobe.

what more dorsal posterior extremity. These extend as furrows posteriorly from the lateral dorsal side of the caudal end of the middle part (fig. 25). As just stated, the posterior extremity is somewhat dorsal to the other parts of the anterior lobe and is directly ventral to the superior lobe. Two deep horizontal furrows constrict the connection between the posterior extremity and the superior lobe.

The superior lobe is just below the saccus vasculosus to which it is closely attached, and is almost three times as wide as the posterior extremity of the anterior lobe (fig. 25). Its median part is about as long as the posterior extremity of the anterior lobe, but projects beyond it caudally and laterally. The caudal end extends somewhat dorsally. The lateral parts, or wings, of the superior lobe project somewhat upward and forward.

The inferior sacs extend laterally from a constricted median connection. They are much smaller than the wings of the superior lobe, but, viewed from above, give the appearance of two lesser caudal wings. From their median connection a short slender canal extends in a slightly oblique direction upward and forward to connect with the anterior lobe (fig. 25).

Fig. 2 Sagittal section of the hypophysis of an 8 mm. embryo. × 40 (H. E.C. 210). *H*, hypophysis; *I*, infundibulum; *Mo*, epithelium of mouth; *Mm*, median mass connecting the premandibular somites; *N*, notochord; *Po*, post optic groove.

Fig. 3 Sagittal section of the hypophysis of a 15 mm. embryo. × 40 (H.E.C. 228). For abbreviations, see figure 2.

Fig. 4 Sagittal section of the hypophysis of a 19 mm. embryo. × 40 (H.E.C. 138). For abbreviations, see figure 2.

Fig. 5 Sagittal section of the hypophysis of a 22 mm. embryo. × 40 (H.E.C. 231). *AL*, anterior lobe; *Med*, median connection of inferior lobes; *SL*, superior lobe; other abbreviations as in figure 2.

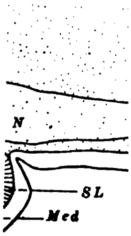
Fig. 6 Sagittal section of the hypophysis of a 28 mm. embryo. × 40 (H.E.C. 234). *St*, hypophyseal stalk; other abbreviations as in figure 5.

Fig. 7 Sagittal section of the hypophysis of a 34 mm. embryo. × 40 (H.E.C. 362). For abbreviations see figure 6.

Fig. 8 Sagittal section of the hypophysis of a 40 mm. embryo. × 40 (H.E.C. 370). *P*, parachordal plate; *T*, trabeculae; other abbreviations as in figure 5.

Fig. 9 Sagittal section of the hypophysis of a 50 mm. embryo. × 40 (H.E.C. 444). *C*, interhypophyseal canal; for other abbreviations, see figure 8.

Fig. 10 Sagittal section of the hypophysis of a pup, (reconstructed from transverse sections). × 40. For abbreviations, see figure 8.



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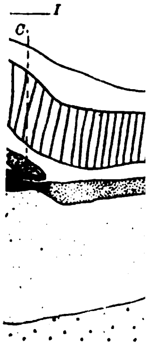

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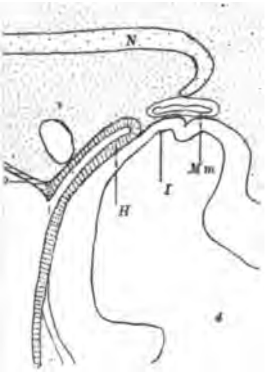

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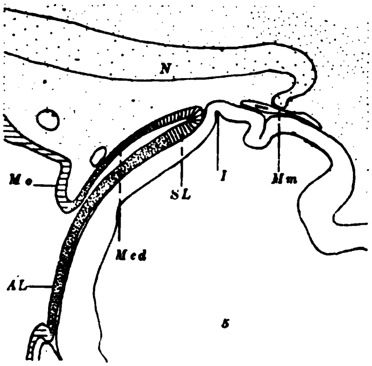

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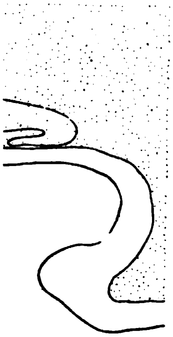

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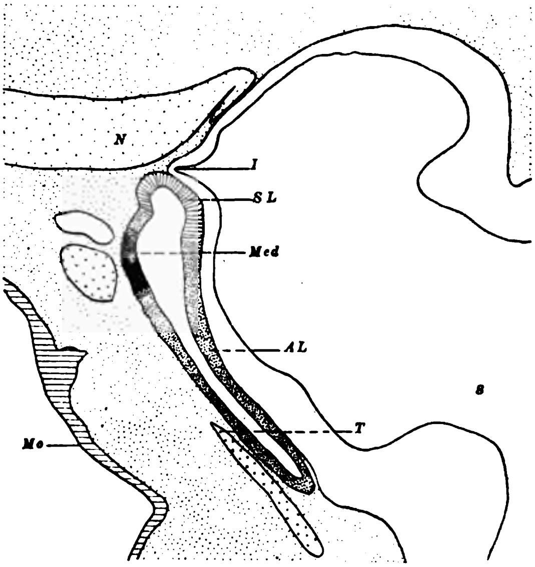

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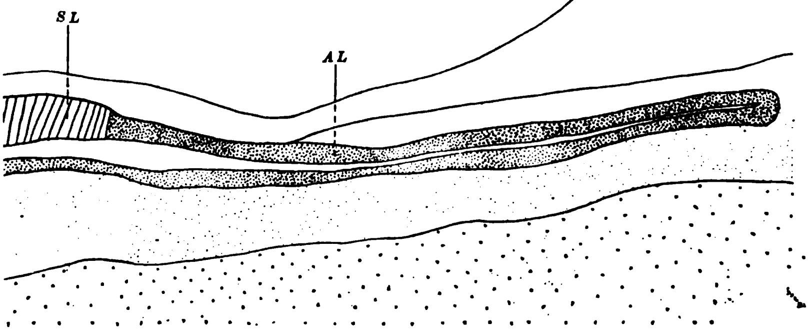

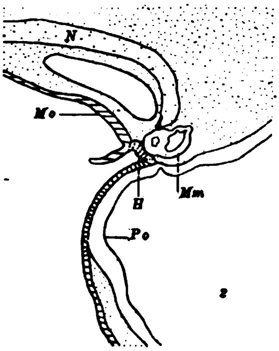
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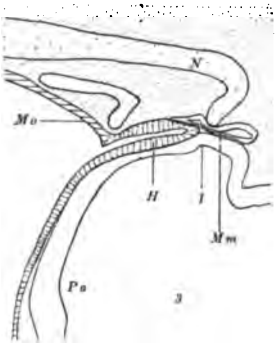
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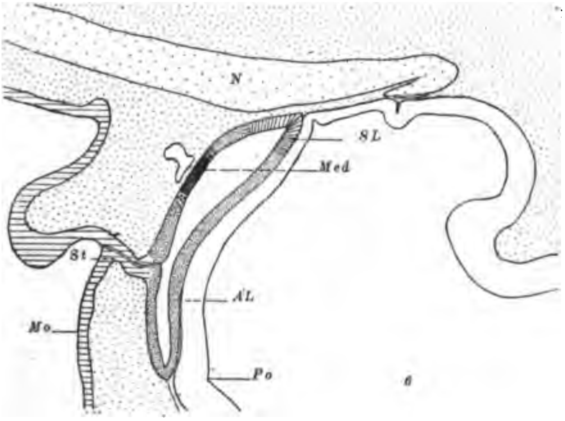
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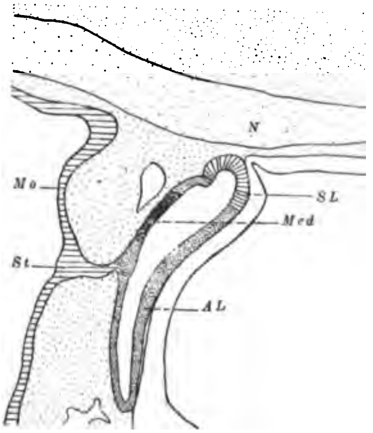
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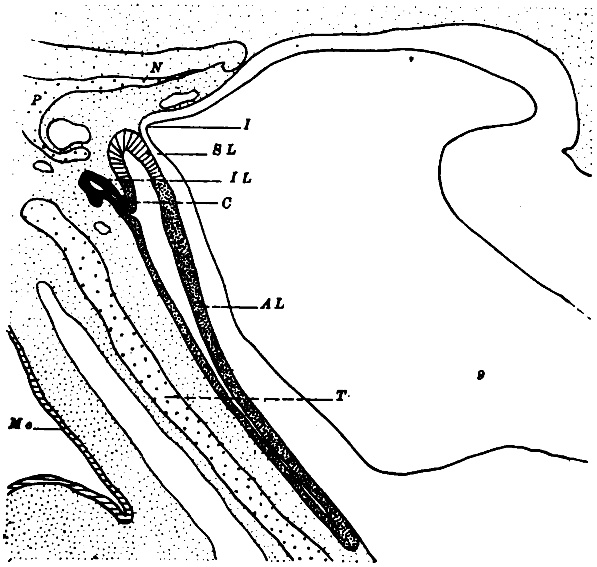
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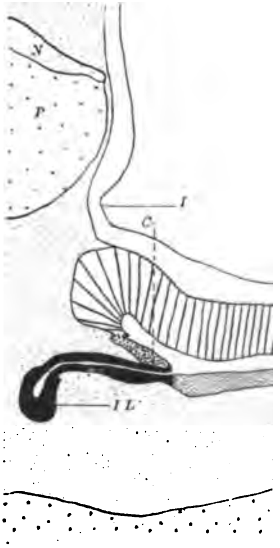
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The position of the adult hypophysis has been described by Sterzi ('09) and others. The long tongue-like anterior lobe lies on the median ventral wall of the inferior lobes of the brain. Its anterior end extends forward almost to the optic chiasma. The superior part is placed posterior to this and in a more dorsal plane. It extends as far caudally as the saccus vasculosus. The inferior sacs of Squalus do not extend as far ventrally as has been described for other selachians. But they are ventral to the superior lobe and extend farther caudalward (fig. 10). From their middle connection a slender canal joins them to the ventral side of the caudal end of the anterior lobe.

2. Early development of the hypophysis

Recent work on the development of the hypophysis in elasmobranchs shows that it arises at an earlier period than was formerly believed. Hoffmann ('96) stated that the position of the future hypophysis is well marked in Acanthias embryos of 15 somites but that there is no indication of an evagination even in 8 mm. (50 somites) and 10 mm. embryos. Haller ('96) began his description of the hypophysis in Mustelus in embryos 22 mm. long. At that time, the hypophysis is already a distinct outpouching. More recently Johnston ('09) briefly described the earliest formation of the hypophysis in Acanthias. In an embryo of 24 somites the ectoderm from which the hypophysis develops is readily recognized. He stated that a short anterior lobe, developing later, extends toward the optic chiasma. Also, that the posterior part crowds between the brain and the median mass connecting the premandibular somites. Scammon ('11) mentioned a thickened hypophyseal plate in a 5.2 mm. embryo and a beginning evagination in a 6.2 mm. embryo (50–51 somites).

A median sagittal section of an Acanthias embryo 8 mm. in length[3] is shown in figure 2. The anterior superior end is partially insinuated between the brain and the median mass connecting the premandibular somites, as had been noted by Johnston ('09) in about the same stage. During the time that the anterior part of the hypophyseal evagination is growing between the brain and the median mass connecting the premandibular somites, the posterior portion forms a more or less prominent ridge caudal to the connecting mass of the somites. A model of such an embryo shows as an evagination, the end of which is grooved transversely by the median mass connecting the premandibular somites (fig. 2). This groove may be quite prominent even in 11 to 12 mm. embryos and evidences of it are usually to be found at that time.

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[3] In the description of the figures 'H. E. C.' has reference to the embryos of the Harvard Embryological Collection used for this study.

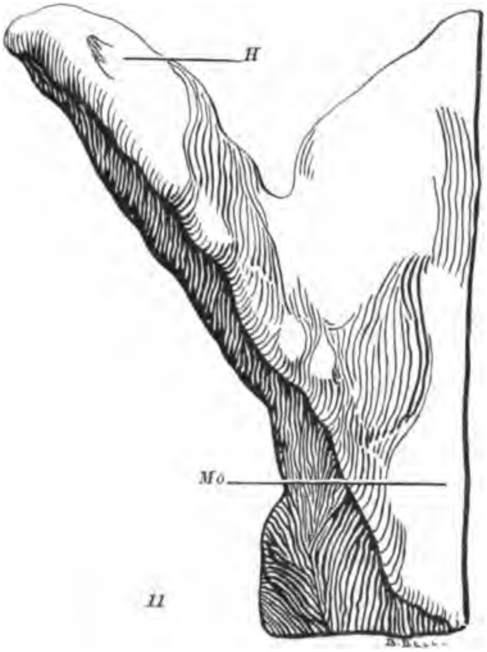
In 8 mm. embryos as well as in younger ones the anterior arm of the hypophyseal outpouching shows differentiation as far forward as the postoptic recess (fig. 2). Scammon ('11) stated that the notochord comes into contact with the early hypophyseal outpouching, and Sterzi ('12) has shown such a contact in Mustelus of 8 mm. (fig. 449). No such contact has been observed in this study of Acanthias.

A mid-sagittal section of a 15 mm. embryo shows that the superior end of the hypophysis has now grown well between the brain and the median mass connecting the premandibular somites. The evagination is more marked and the anterior arm is longer than in younger stages (fig. 3).

A model of the hypophysis of an Acanthias embryo 19 mm. in length is shown in figure 11; this view is taken from the left lateral side. The hypophysis at this stage is an anteriorly and dorsally directed outpouching, concave on its ventral surface where it lies in close relation to the diencephalon (fig. 18). Its superior anterior end extends to the infundibular recess. The opening from the pharynx into the hypophysis is small. A sagittal section at about the median line (fig. 4) shows that the thickened anterior (ventral) wall of the hypophysis extends forward as far as the postoptic groove as Johnston ('09) has figured it.

Fig. 11 Left lateral view of a reconstruction of the hypophysis of a 19 mm. embryo. × 130. *Mo*, lining of mouth; *H*, hypophysis.

Fig. 12 Left lateral view of a reconstruction of the hypophysis of a 21 mm. embryo. × 100. *a*, anlage of anterior end of hypophysis; *H*, hypophysis; *IL*, anlage of the inferior lobes; *Mo*, lining of mouth.



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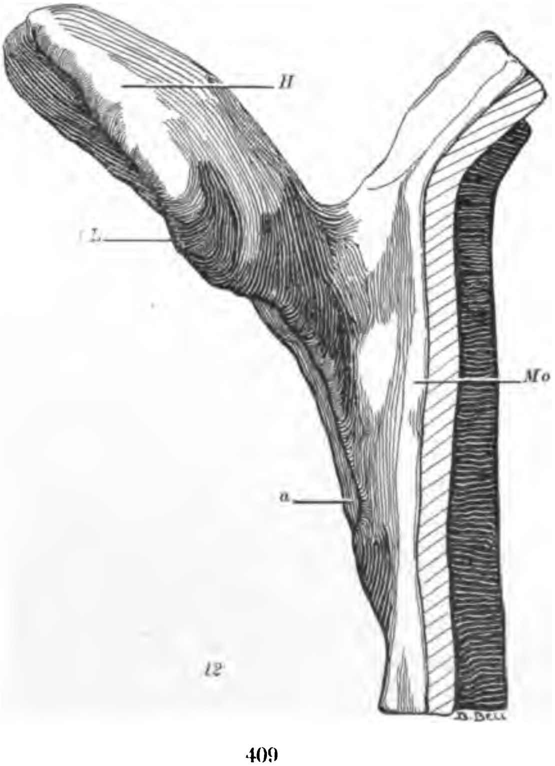

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Several changes have taken place in the hypophysis of a 21 mm. embryo. It is still concave, both laterally and dorso-ventrally in its ventro-anterior surface (fig. 12). The thickened anterior wall of the hypophysis, reaching almost to the preoptic groove, is now distinctly evaginated. Scammon ('11) mentioned this closing off of the anterior part in a 20.6 mm. embryo and Sterzi ('12) described the formation of this 'rostral diverticulum' in 20 to 24 mm. embryos. The lateral side of the anterior outpouching is sharply demarcated by the formation of the stalk connecting the hypophysis to the mouth (fig. 19). The anterior end at this stage is almost half as wide as the posterior, from which most of the hypophysis is developed. The opening from the mouth into the early anlage is located as before, but is smaller now. A view of a model from the oral side shows that the constriction of the front and lateral sides of the anterior end of the anterior lobe has begun.

In a 22 mm. embryo the hypophysis (fig. 13) is not as concave as in younger stages. The anterior part shows laterally more marked constriction from the stalk which connects it to the buccal cavity. The anterior end is also markedly constricted. The opening into the hypophysis extends now from this anterior constriction to the posterior (caudal) margin of the opening into the first outpouching. The opening into the first evagination is very small and connects the pouch with the stalk (fig. 5). The posterior end is wider transversely than before. On its dorso-lateral surfaces are small ridges (fig. 13), the anlagen of the inferior sacs. On the ventral surface of the posterior part are two slight lateral furrows which are beginning to separate the inferior sacs from Rathke's pouch (fig. 20). These furrows are present in a 20.6 mm. embryo, as Scammon ('11) stated. The lateral pouches or inferior sacs appear as dilated cavities at either side. The grooves or furrows are as yet shallow and indistinct. In an 18 mm. embryo, Scammon ('11) noted the beginning of the division, by slight furrows, of the posterior portion into a median and two lateral parts. Some embryos do indicate the beginning division of the inferior sacs about that time but the furrows are not as prominent as the dilated cavities.

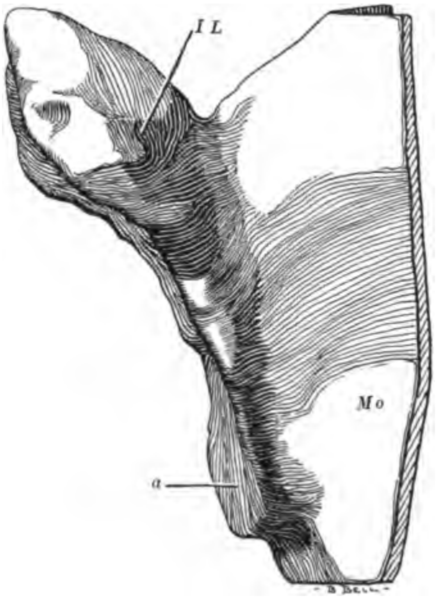


Fig. 13 Left lateral view of a reconstruction of the hypophysis of a 22 mm. embryo. × 100. For abbreviations, see figure 12.

In a model of a 28 mm. embryo (fig. 14) the entire anterior portion is well closed off. It extends anteriorly almost to the postoptic recess. From its caudal surface a slender stalk connects the hypophysis with the mouth (fig. 6). The ventral end of the anterior lobe is somewhat wider than the mid part which connects it with the very much wider posterior portion or Rathke's pouch (fig. 21). Distinct furrows on the anterior (ventral) side partially separate the lateral anlagen of the inferior sacs from the original Rathke's pouch. A slight ridge on the caudal (superior) surface joins the two inferior sacs (fig. 14). In a median sagittal section (fig. 6) a shallow groove marks the connection between them. This connection is dorsal (caudal) to the stalk joining the hypophysis to the mouth. On the lateral dorsal surface at the posterior end of Rathke's pouch two very slight outpouchings indicate the position of the developing superior lobe (fig. 14).

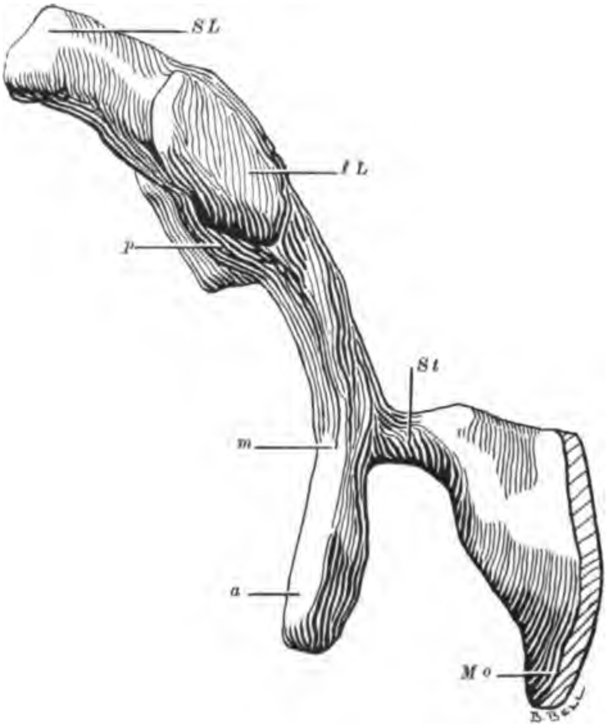


Fig. 14 Left lateral view of a reconstruction of the hypophysis of a 28 mm. embryo. × 100. *a*, *p*, anterior and posterior extremities of anterior lobe; *IL*, inferior lobe; *m*, middle part of anterior lobe; *Mo*, wall of mouth; *St*, hypophyseal stalk; *SL*, anlage of superior lobe.

3. *Later development of the hypophysis*

All the main outpouchings of the hypophysis are present in the 28 mm. embryo. The inferior lobes are quite prominently marked off, the anterior lobe shows two widened extremities and the superior lobe has just begun to evaginate. The anterior end of the hypophysis in a 33 mm. embryo is curved slightly forward. The anterior lobe is not so markedly concave anteroposteriorly as in earlier stages (fig. 7). The ventral (anterior) furrows separating the inferior lobes from the posterior medial portion, are now quite deep (fig. 15). On the caudal side a distinct ridge extending between the inferior lobes indicates their

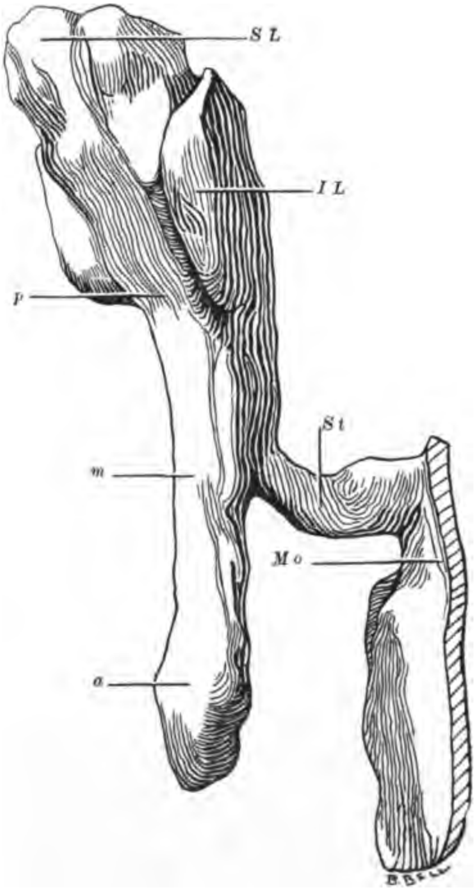


Fig. 15 Left lateral view of reconstruction of the hypophysis of a 33 mm. embryo. × 100. For abbreviations, see figure 14.

early connection with each other. On the posterior tip of the hypophysis two lateral grooves mark the beginning constriction of the superior lobe (fig. 22), the outpouching of which was seen in the previous stage. The stalk connecting the hypophysis to the pharynx is smaller than in earlier stages.

The anterior lobe of the hypophysis, particularly its anterior part, has increased in length in a 40 mm. embryo. The stalk joining the hypophysis with the mouth has disappeared, with

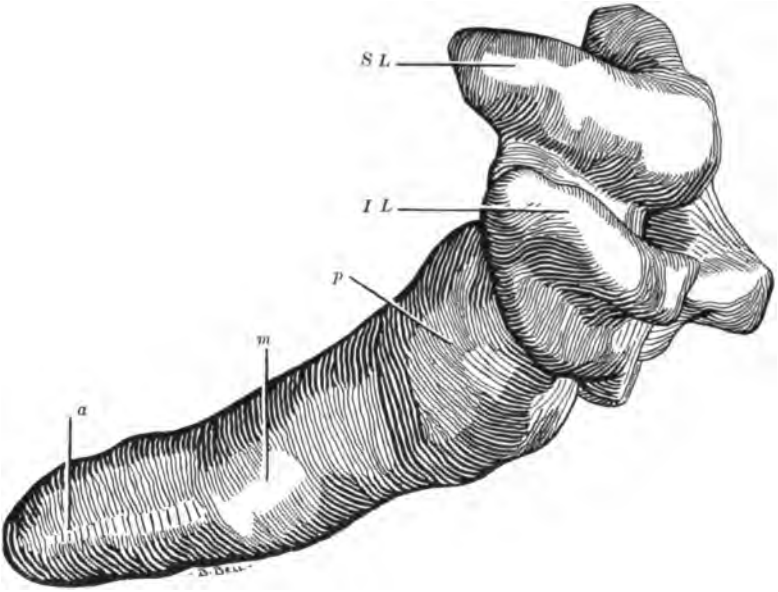


Fig. 16 Left lateral view of a reconstruction of the hypophysis of a 48 mm. embryo. × 100. For abbreviations, see figure 14.

the exception of a cone-shaped mass of cells connected with the oral epithelium, and an irregular area in the floor of the hypophysis which represents the remains of the former attachment (fig. 8). The furrow uniting the two inferior lobes across the middle line is more marked, and the superior lobe also is prominent. The anterior end of the hypophysis now extends forward and downward. The straightening out of the head bend in the development of the embryo has probably helped to bring about this change.

More marked changes have taken place in a 48 mm. embryo (fig. 16). The anterior end of the anterior lobe is wider than in earlier stages. A short and narrow middle part connects this portion with the caudal extremity which is considerably wider (fig. 23). The inferior lobes are attached to the, now, ventral (caudal) side of this part. The ridge connecting the two inferior lobes has become very pronounced but still opens widely into the anterior lobe (fig. 16). The furrows separating the inferior lobes from the anterior are much deeper and wider. The inferior lobes have enlarged in their dorso-ventral and in their transverse diameters, and they extend laterally beyond the posterior extremity of the anterior lobe. Marked development has taken place in the superior lobe. The lateral furrows separating it from the anterior one are deeper, and the cranio-caudal length of the lobe has increased so that there is a projection caudally beyond the anterior lobe. The antero-lateral ends of the superior lobe have grown forward.

The median connection of the inferior lobes is constricted from the posterior (ventral) part of the anterior lobe in a 50 mm. embryo. The inferior lobes are directly ventral to the superior lobe. There remains a short slender tube in the mid-line connecting the inferior lobes to the anterior (fig. 9). The duct connecting them to the anterior lobe extends almost straight anteriorly.

A median sagittal section of an 86 mm. embryo (fig. 27, G) shows an increase in the length of the hypophysis. The inferior lobes lie more caudally and the duct joining them to the anterior lobe is longer.

In a 95 mm. embryo the anterior lobe has increased greatly in length (fig. 17). A median ventral sulcus has appeared and the anterior third of this lobe is quite wide. A middle narrow portion, almost circular in cross section, connects the anterior extremity to a wider posterior end (fig. 24). The caudal extremity is connected dorsally with the superior lobe. The inferior lobes are continuous across the median line. The connection between the inferior lobes and the anterior one is a small tube which extends almost straight forward to join the inferior surface of the caudal end of the anterior lobe just below where this opens into the superior one (fig. 10). The inferior lobes have enlarged in their cranio-caudal axis. The lateral parts of the superior lobe have increased in their cranio-caudal diameters and extend forward beyond the median part. The latter has grown caudalward and lies just dorsal to the tube joining the inferior and anterior lobes.

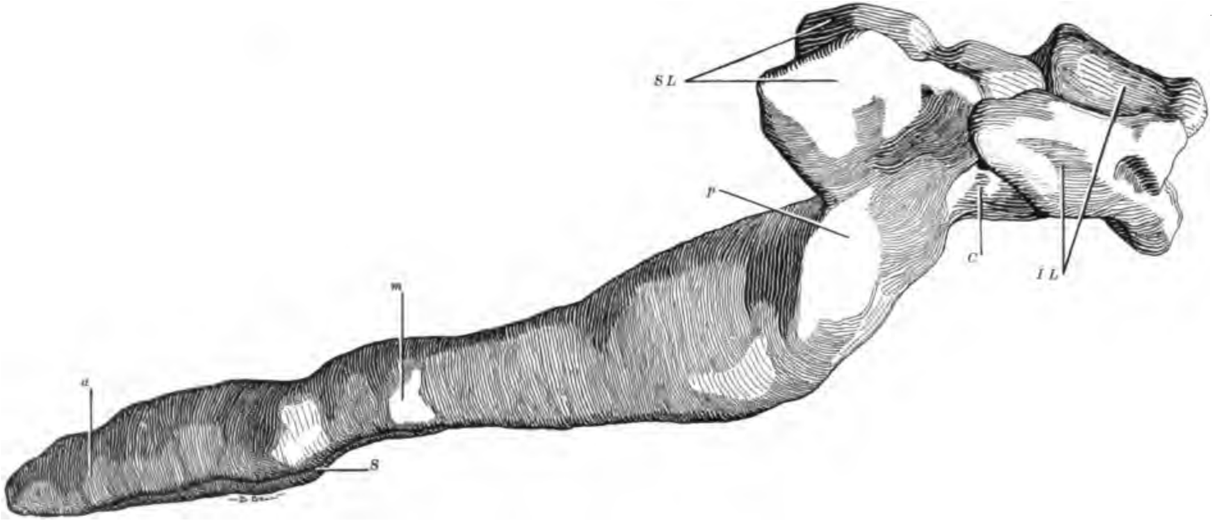
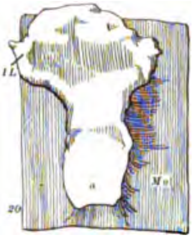


Fig. 17 Left lateral view of a reconstruction of the hypophysis of a 95 mm. embryo. × 75. *C*, interhypophyseal canal; *S*, median ventral sulcus; other abbreviations as in figure 14.

4. The hypophysis of the adult

A detailed description of the pup stage has been given (p. 400) as typical of the morphology of the adult condition (fig. 1). A drawing of a dissection of the hypophysis of an adult will show the position and relation of some of the parts more clearly (fig. 26). The anterior end extends almost to the optic chiasma, as noted by Sterzi and others. From a study of the models of the 'pup' stage and of sections of adults it appears that the middle part of the anterior lobe is more than a mere constriction separating a larger rostral from a smaller caudal part as Sterzi ('09) described. In the pup this middle part is only a little shorter than either extremity, while in the adults it is much shorter. It is, however, distinctly marked. In some cases there are cystic outgrowths from the floor of this part; in others the walls and floor are quite regular and therefore are distinctly different from either extremity. The middle part is smaller than either extremity in its dorso-ventral and lateral diameters in pups (figs. 24, 25) and in adults, but the changes in diameter from either end to the middle part in adults takes place gradually and the parts are not so definitely marked, except in those cases in which no glandular outgrowths occur from the middle region which is much more prominent in sections and in wax reconstructions than is shown in dissections. The superior lobe projects some distance laterally on the ventral side of the vascular sac. It is difficult to make out the lateral limits of this part in the dissected specimen, as it seems to be continuous with the ventral surface of the vascular sac (fig. 26). In transverse sections, however, the lateral extent of the wings of the superior lobe is clear. The superior lobe is convex dorsally and closely attached to the saccus vasculosus. The latter dips down on either side of the caudal end of the anterior lobe and so partially separates the lateral wings of the superior from the anterior lobe.

The inferior lobes no longer resemble the sac-like structures with cystic outpouchings of the pup stage. Both superior and inferior walls have developed a mass of tubular-like glands. None of these was observed extending cranialward, as described by



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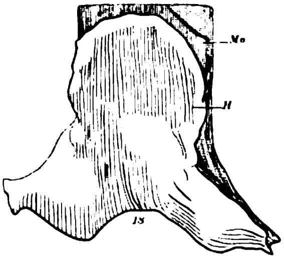

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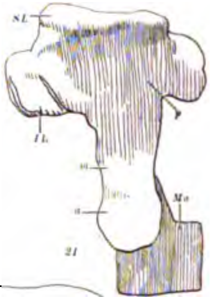

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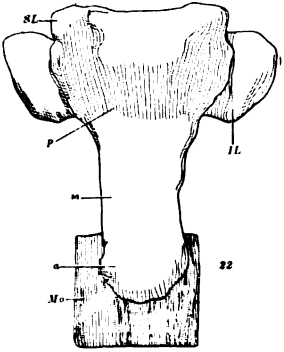

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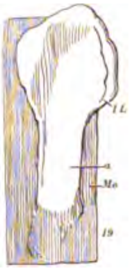

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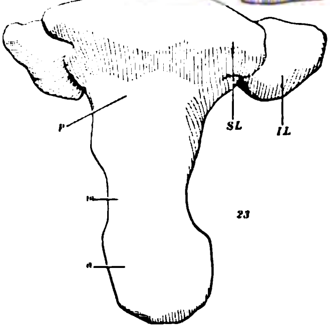

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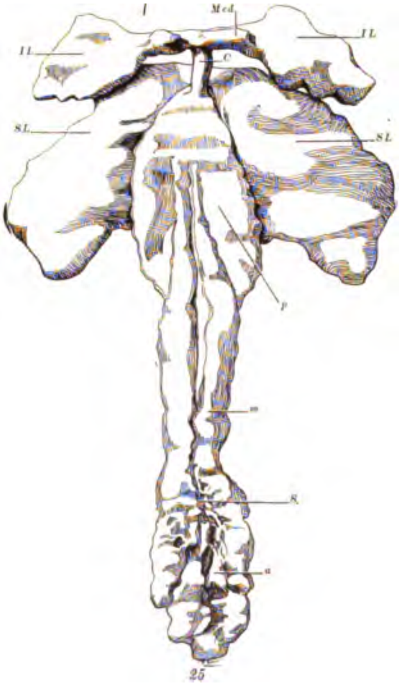

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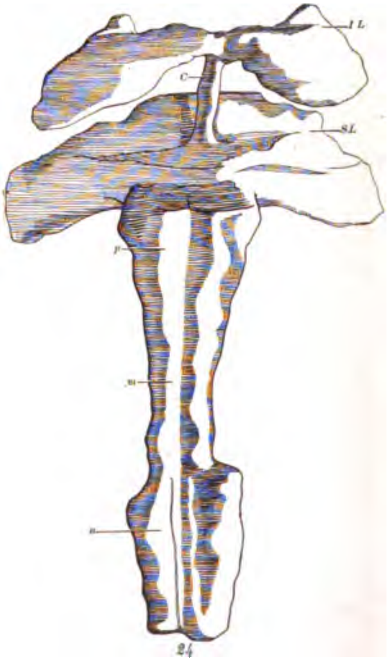

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Figs. 18–20 Antero-ventral views of the reconstructions shown in figures 11 to 13. Figure 18, × 50; figures 19, 20, × 40.

Figs. 21–23 Anterior views of the reconstruction shown in figures 14 to 16. × 40.

Figs. 24–25 Ventral views of the reconstructions shown in figures 17 and 1. Figure 24, × 40; figure 25, × 25. For abbreviations, see figure 1.

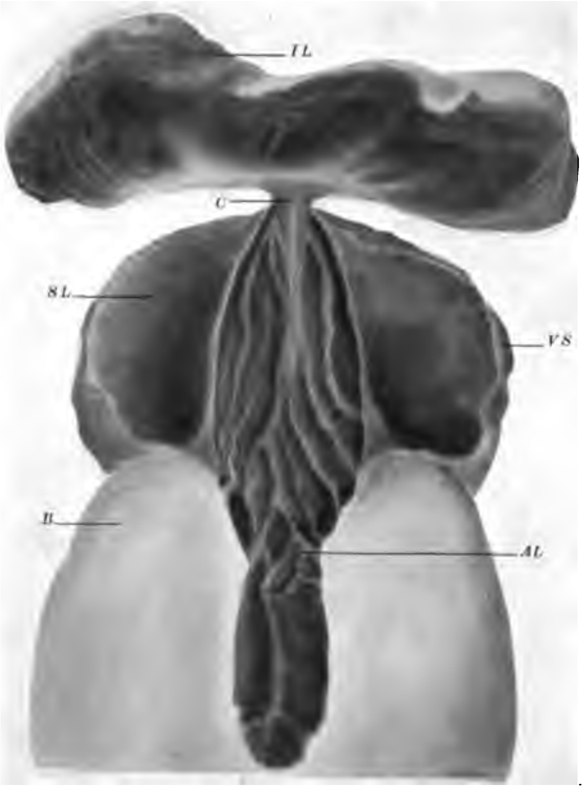


Fig. 26 Drawing of a dissection of the hypophysis of an adult Acanthias from ventral side. × 20. *AL*, anterior lobe; *B*, inferior lobes of the brain; *C*, interhypophyseal canal; *IL*, inferior lobes of the hypophysis; *SL*, superior lobe of the hypophysis; *VS*, vascular sac.

Haller in Mustelus. From their median connection a long slender tube extends to the caudal end of the floor of the anterior lobe. As seen in figure 26, the median connecting portion of the inferior lobes may be large. In some specimens this part, as well as the lobes, shows a mass of tubules. The caudal ends of the inferior lobes are surrounded by cartilage.

The shifting of the hypophysis with reference to its position in the body and the development of different parts is brought out very clearly in figure 27. Sewertzoff ('99), in his studies on the development of the skull, made use of such a figure to show the interrelation of development of the brain and skull. He made an outline drawing of the skull and brain of an embryo, choosing an arbitrary magnification. He then made drawings of different sized embryos of such a magnification that points, corresponding to two arbitrarily chosen in the first drawing, would coincide. In figure 27 of this paper, the same scheme was adopted. The magnification of the first drawing was such as to avoid as much as possible the confusion of lines. The points chosen were the extreme anterior end of the notochord and the axis of the notochord at the level of the first spino-occipital nerve. All the other drawings were then made so that these two points—the extreme anterior end of the notochord and the axis of the notochord at the level of the first spino-occipital nerve—should coincide with those of the first drawing. The outlines of the hypophysis were then drawn, using a line between the two points as a base.

The objections to such a drawing are readily apparent. There are, of course, individual variations in the embryos. Also, these points are probably continually changing during development. For a comparative study, however, the variations can be no great objection and the points chosen are probably as reliable as any. A series of embryos from 11.5 mm. in length to the pup were drawn in this manner (fig. 27). One can see at a glance in all these stages the relative position of the hypophysis with reference to the anterior end of the notochord. Also, as was pointed out in the description of the different embryos, what is first the dorsal wall becomes in later stages the ventral, while the ventral or anterior becomes dorsal. The early superior end of the evagination shifts more and more caudalward with reference to the rest of the hypophysis, until, in the pup, the superior lobe, which develops from the superior dorsal end, is caudal in position. The inferior lobes, which develop from the sides of the superior end and are on the same horizontal plane as the superior position, take a position ventral to the superior lobe in the late embryo and adult. The furrows separating these inferior lobes, described as appearing in the 20 to 22 mm. embryos

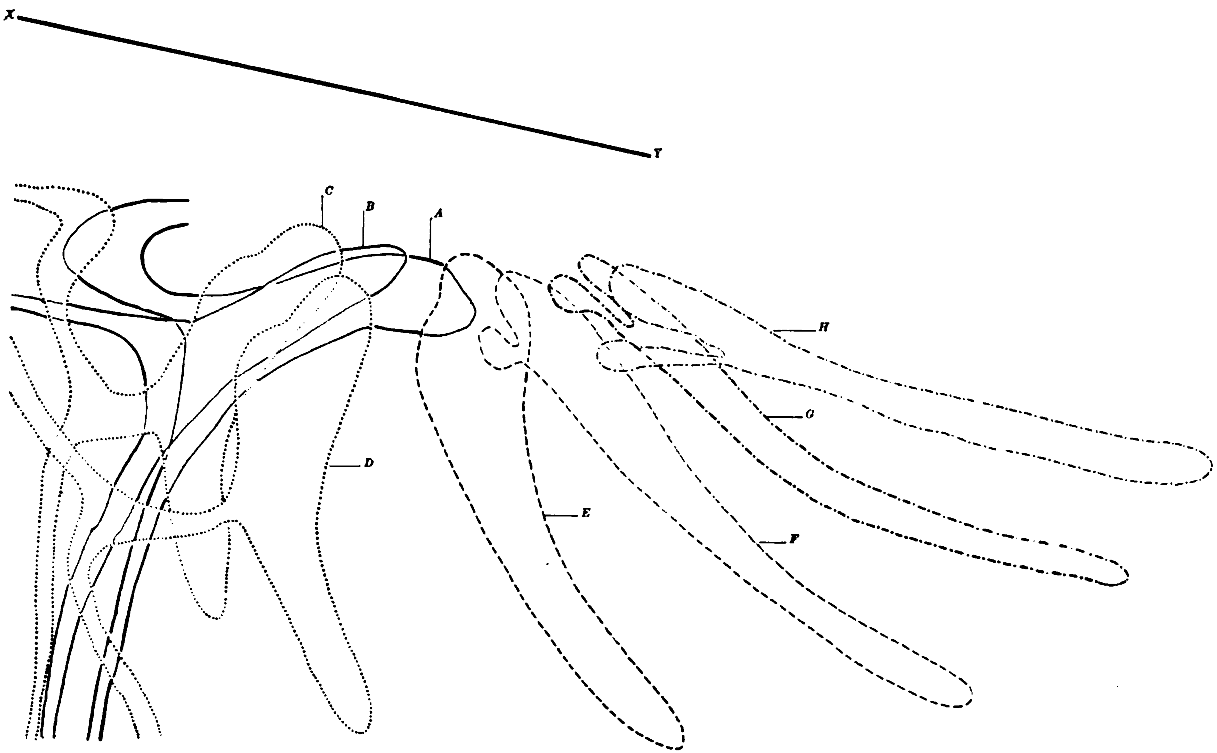


Fig. 27 Composite drawing of median sagittal sections of the hypophysis of Acanthias embryos. *A*, 11.5 mm. embryo; *B*, 18 mm.; *C*, 34 mm.; *D*, 37 mm.; *E*, 40 mm.; *F*, 50 mm.; *G*, 86 mm.; *H*, pup. *X–Y*, base line drawn from the anterior end of the notochord to the axis of the notochord at the level of the first spino-occipital nerve.

on the ventral (anterior) side of the hypophysis, are later (50 mm. embryos) on the dorsal side. The anterior lobe, first directed almost vertically, grows ventrally and later extends more and more anteriorly until it is directed horizontally (cranio-caudally).

The comparative growth of the different portions is also made clear. The anterior lobe comprises all of the original outpouching and also the anterior tongue which evaginates later. The increase in length of the anterior lobe, particularly its anterior extremity, is marked. The inferior lobes, developing from the sides of the posterior portion of the early evagination, become continuous across the posterior side (34 mm. embryos) and finally constrict entirely except for a short duct connected with the anterior lobe. The inferior lobes increase greatly in size, but, in the adult, are largest transversely. The superior lobe, developed from the superior dorsal part, spreads far out transversely and later enlarges in its dorso-ventral axis.

The posterior extremity of the anterior lobe is then developed from the first outpouching. A little later (21 mm.) the part anterior or ventral to the original outgrowth evaginates, forming largely the middle narrower portion of the anterior lobe. The anterior extremity of the anterior lobe develops at the extreme anterior (ventral) end of this. The stalk connecting the hypophysis with the mouth is attached to the middle narrower portion. The connection between the inferior lobes develops caudal (dorsal) to this but arises from a part which later becomes the floor of the posterior extremity of the anterior lobe. From this description it is seen that the inferior lobes, developing from the posterior end of the hypophyseal anlage, and the superior lobe, from its extreme dorsal (anterior) end, are derived from a part of the anterior lobe. Such an explanation of the development of the parts is well borne out by a study of the models as well as of the various sections of embryos.

In comparing figures 6, 7 and 8 it is seen that the hypophyseal stalk in a 40 mm. embryo is attached nearer the caudal end of the hypophysis than it is in younger ones. This does not agree with what Haller has described for Mustelus when he found the place of attachment of the hypophyseal stalk near the anterior end. In a 90 mm. Mustelus this place of attachment can be recognized by the very thin floor, and in the adult, by the opening into the subdural space (Haller '96, figs. 12 and 40).

The cavity in the hypophysis of elasmobranchs has been variously described as barely distinguishable, slit-like and large. It *is* large in some adult specimens of Acanthias. In the anterior lobe there is a distinct increase in the size of the cavity during its development. Table 2 will show the actual increase in the depth of the cavity. The measurements here given were taken in the caudal part of the anterior extremity of the anterior lobe.

TABLE 2

Showing depth of the hypophyseal cavity

SPECIMEN	THICKNESS OF ROOF IN MICRA	DEPTH OF CAVITY IN MICRA	THICKNESS OF FLOOR IN MICRA
Embryo 34 mm.	31	37	25
50 mm.	31	13	18
86 mm.	35	12	22
Pup	56	90	50 including glandular outgrowths
Adult	56	470	480 including glandular outgrowths

The increase in size of the lumen from the pup to the adult is thus seen to be considerable. From the table and from comparison with figures 2 to 10 it is seen that the increase in size of the lumen is not gradual through all the stages. For example, in the 50 mm. embryo the lumen is actually smaller than in a 34 mm. embryo. It is only from the early pup stage on that the lumen increases to any considerable degree. The increase in size of the lumen of the inferior lobes is even more marked. The cavity in the superior lobe is never large. It is very small in a 48 mm. embryo where the lateral wings are first prominent. In a 95 mm. embryo this cavity is small, but is still distinct. The lumen of the middle portion of the superior lobe extends transversely and forward in the lateral wings of the superior lobe in pups. There is no evidence at any time of an extension of the lumen into any of the glandular columns of this part. In the adult all trace of a central cavity has disappeared, unless the small secretion spaces, to be described later, are remains of the original lumen. Occasionally there is a more or less prominent median dorsal extension of the cavity of the anterior into the superior lobe. In a very few cases this dorsally-extending cavity is continuous with a secretion space lying laterally.

Table 3 shows the increase in size of the hypophysis; also, the length of the hypophysis at the median line, the greatest width of the anterior extremity of the anterior lobe, and the greatest width of the superior lobe. The table shows there has been a constant increase in length which is rather more rapid in earlier stages. The increase in width of the superior lobe is a gradual one in the beginning. The anterior lobe, however, grows very little at first. This part doubles in width in its growth between the 22 mm. and the pup stages. It almost doubles again in its later growth.

TABLE 3

Showing increase in size of the hypophysis

SIZE OF SPECIMEN	LENGTH OF HYPOPHYSIS IN MM.	GREATEST WIDTH OF SUPERIOR LOBE IN MM.	GREATEST WIDTH OF ANTERIOR EXTREMITY IN MM.
Embryo 22 mm.	0.69		0.23
28 mm.	0.81	0.40	0.22
33 mm.	1.04	0.96	0.30
48 mm.	1.00	1.07	0.35
95 mm.	1.34	1.30	0.40
Pup	3.54	1.91	0.53
Adult	6.00	4.00	1.00

From a brief study of a few Torpedo embryos, I am inclined to believe, with Sterzi, that the lateral lobes, described in this form by Gentes ('08) and others, are comparable with the inferior lobes of Acanthias. The embryos examined show no prehypophyseal body such as Chiarugi ('98) described in this genus, nor is there anything comparable to the prehypophyseal body found in the embryos or adults of Acanthias.

HISTOLOGY AND HISTOGENESIS

1. *Histology of the adult hypophysis*

a. Anterior lobe. The deep median sulcus in the floor of the anterior lobe has been noted above. It has been observed by Sterzi and others in various selachians. The furrows noted in the floor of the anterior extremity in the pup are evidence of the beginning formation of tubules. Haller observed the tubules on the ventral wall of the anterior part in Mustelus, Gentes observed them in Torpedo, and Sterzi in Acanthias. Tilney ('11) stated that there are many vesicles in the upper and lower walls of the juxta-neural (anterior) part in Acanthias. Haller figures cyst-like glands in the roof of the anterior lobe of Mustelus. Some adult specimens of Acanthias show glandular outgrowths in the superior wall of the anterior lobe, especially in the dorso-lateral parts of the anterior extremity. As Sterzi ('09) observed, glands probably develop from the anterior lobe throughout life and, finally, even from the dorso-lateral walls and roof, and glands project ventrally from the floor of the anterior lobe. A model of some of the latter shows that branches are given off at all angles from the first large tubule extending ventrally and that secondary and tertiary outpouchings occur from the branches. The tubules anastomose among each other and with those from other glands both cranially and caudally, but not across the median sulcus with the glands of the other side. The lumina may be continuous through the anastomosing tubules. A cast of the lumina shows frequent enlarged cavities from which small openings lead to the cyst-like cavities found in some of the secondary and tertiary tubules. No such glands were observed in any other part of the anterior lobe. If any glands be present in the roof of the anterior end they are simple and cystic or acinar in character. In some specimens, probably older animals, there are large tubular glands in the floor and lateral walls and even in the roof of the caudal extremity. The floor of the middle part occasionally shows cystic glands. The walls of the tubules are two or three cells thick and the columnar cells forming them are at right angles to the surface (fig. 28). There is a periph-

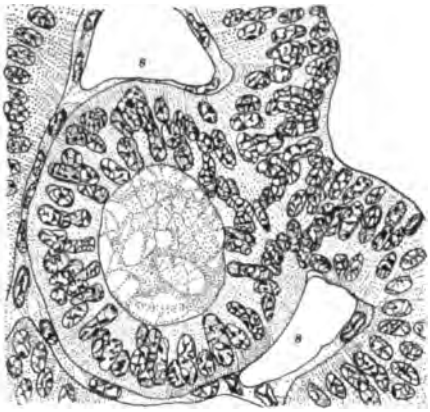


Fig. 28 Transverse section of the floor and a gland of the anterior lobe of an adult. × 400. s, sinusoid.

eral zone of cytoplasm which is very finely granular and contrary to the statement of Tilney, it is acidophilic. Haller observed that the peripheral zone of cytoplasm in Mustelus stained intensively with borax carmine and Stendell stated that the peripheral layer of cells is acidophilic. Sterzi ('09) found that the cytoplasm of the cells in the floor of the caudal part of the rostral lobe is not granular. However, there are glandular outgrowths here in some adults and these walls have the same character as the other glandular parts, except in the mid-ventral line where no outgrowths are found, and here the wall is composed of low epithelial cells. The nuclei are oval and crowded nearer the inner free surface. They have a finely granular chromatic network. The roof of the anterior lobe is also composed of several layers of cells. If no glands are present, the nuclei here are very irregularly placed, some of them lying parallel to the surface along the inner free side, others being placed at various angles to the surfaces. The cytoplasm is very scant. The roof comes into close relation with the overlying brain, but a thin connective tissue layer containing small blood vessels and capillaries separates them. One cannot speak of a fusion of the roof of this lobe with the brain tissue above, as noted by Tilney.

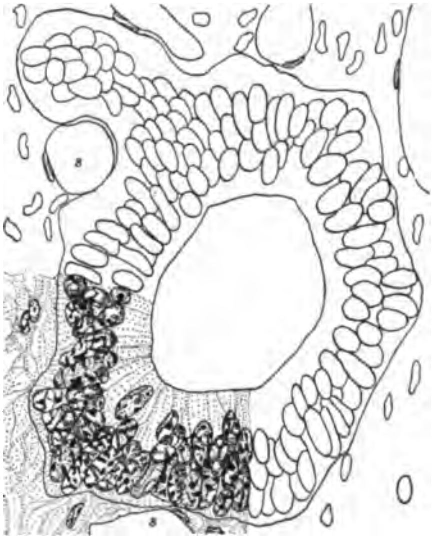


Fig. 29 Transverse section of a gland of the inferior lobe of an adult. × 400.

b. Inferior lobes. The inferior lobes are large glandular structures, from the walls of which are many tubular outgrowths. These tubules are especially numerous on the ventral surfaces of the inferior lobes although there are some on the roof also. Two or three layers of columnar cells form the walls. In this case a wider cytoplasmic zone lies along the inner free surface (fig. 29). The cytoplasm is clear and the cell membranes stand out distinctly. The cells are very faintly acidophilic. The nuclei here, as in the anterior lobe, are oval in outline, and the long axis is always at right angles to the free surfaces. The chromatin here also is in a fine network with a more or less definite layer along the nuclear membrane.

c. Superior lobe. As has been observed in all selachians, there is a great glandular outgrowth from the roof of the superior lobe. As Sterzi has noted, these glands are not tubular but solid. The floor of this portion shows no glandular outgrowth. It is made up of several layers of columnar cells. The roof is several cell-layers deep and from it many thick columns extend upward.

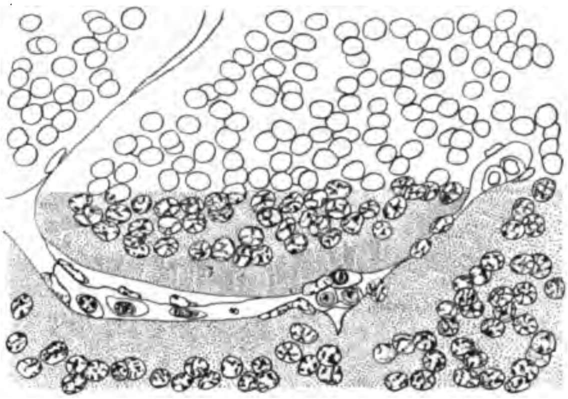


Fig. 30 Sagittal section of the glandular cords of the superior lobe of an adult. × 400.

The nuclei of the columns are spherical in shape, crowded close together in the center, and contain a light chromatin network and a nucleolus. Between these columns are numerous sinusoids. Along the periphery of the columns is a thick layer of granular cytoplasm, the granules being small and closely crowded (fig. 30). The cytoplasm frequently takes a distinctly acid stain. In some eosin-methylene blue preparations the cytoplasm of the superior lobe along the periphery of the columns is stained blue. With iron hematoxylin this same zone sometimes retains the hematoxylin longer than does the chromatin. The granules of this part often stain an intense blue with Mallory's phosphotungstic acid stain. On either side of a granular area which stains very deeply there may be a clear area where the secretion possibly may be forming or has just been given up. I cannot, therefore, agree with Tilney ('11) who finds only eosinophilic cells in the superior lobe and basophilic in the anterior lobe. Besides, as has been noted, in many cases the cells of the anterior lobe take eosin quite as readily. Stendell ('13) thought that the division of chromophobic and chromophilic portions as described by Sterzi and others might not be true in all cases. In the adult Acanthias, it seems to me this distinction cannot be sharply drawn, at least not with all stains. The cytoplasm of the superior lobe stains readily, and indeed, sometimes more deeply than that of the anterior lobe. The nuclei, on the other hand stain less deeply than those of the anterior lobe. Those of the inferior lobe frequently stain very lightly. Sterzi has stated that the cytoplasm of the superior lobe stains with difficulty.

d. Connective tissues, blood vessels and nerves. In very small embryos there are only occasional connective tissue cells lying between the hypophysis and the brain and vascular sac above, and these cells are along the dorso-lateral walls. Beginning in 33 mm. embryos, however, there is a thin layer of mesenchyma between the dorsal (posterior) end of the hypophysis and the anlage of the vascular sac. In 50 mm. embryos small blood vessels are found between the superior lobe and the saccus vasculosus (fig. 37). At this time, also, a thin layer of mesenchyma separates the hypophysis from the brain floor. In the superior lobe of the adult occasional small strands of connective tissue are found in the center of the core of nuclei of the columns.

They anastomose with the connective tissue between the columns but have no cytoplasmic zone bordering them as do the larger, more vascular connective tissue strands between the columns.

In the pup, there are small capillaries in the connective tissue over the anterior lobe of the hypophysis. On the ventral side there is considerable connective tissue between the developing tubules. The few capillaries here are not large. The capillaries between the columns of the dorsal lobe are large and numerous. These capillaries or sinusoids are to be found in the interstices between the cell columns and also between the ends of the columns and the overlying vascular sac. In the adult the capillaries over the anterior lobe are somewhat larger. There has been some increase in the size and number of the capillaries between the tubules of the anterior lobe and the same is true in the superior lobe between the cell columns.

Nerves have been described in the hypophysis by Edinger ('92), Sterzi ('09) and others. Sterzi described the floor of the brain in the hypophyseal area (above the superior lobe of the hypophysis) as composed of three layers, of which the middle was made up of nerve fibers coming from the caudal end of the inferior lobes of the brain. When these fibers reach the area above the superior lobe, numerous bundles of them go ventrally between the columns. These bundles are large and composed of large nerve fibers. According to Stendell ('12) a distinct lumen, continuous with the lumen of the vascular sac, extends into these bundles in Heptanchus. As Sterzi ('09) showed, these bundles are solid in Acanthias. Sterzi was not able to see any of the fibers ending in the cell columns. In some material stained with Mallory's phosphotungstic acid hematoxylin these fibers are well shown. I can not affirm that they do end in the cell columns between the cells as Sterzi was inclined to believe.

e. Secretions. Haller ('96) stated that the lumina of the superior lobe may contain cell detritus or a secretion. Tilney ('11) observed a colloid-like substance in the vascular sac above the superior lobe. Stendell ('13) described deep acidophilic secretion granules in the cytoplasm of the cells of the 'Zwischenlappen.' These colloid-like secretions were found in the cells lying towards the blood vessels. The adult Acanthias studied show no colloid-like secretion granules, such as Stendell found in the cytoplasm near the sinusoids in Mustelus and Scyllium. Considerable colloid-like secretion, however, is found in the tubules of the anterior lobe, also some in the main lumen of this part. The tubule drawn in figure 28 contains secretion. Some secretion was found in the tubular glands and in the main lumen of the inferior lobes. There are also many spaces in the superior lobe which are partially filled with secretion. The spaces are cylindrical in shape, sometimes as much as 20μ in diameter and 50 to 200μ long. They have no special walls, the cells and nuclei lining them appear to have been crowded aside. Frequently the inner layer of nuclei lies flat along the wall. These spaces never come in contact with the sinusoids, but are always found in the middle of the columns and are surrounded by nuclei which are crowded close together. Aresu (14) has described similar cyst-like spaces in Chimaera, containing a substance which stains lightly with basic stains. That the secretion does not fill the spaces may be due to shrinkage, as has been suggested in the case of the colloid in the thyreoid follicles. In all respects it is similar to that found in the anterior lobe. There is no direct outlet by means of which a secretion can reach the cavity of the vascular sac and thus gain entrance to the ventricles of the brain. The absence of any secretion in the vascular sac also argues against there being a pathway for the secretion to enter it. It would seem probable that another secretion, distinct from this, is formed in the lumen of the tubules. As stated in the description of the histology of the adult hypophysis, the granular cytoplasm is always found on the peripheral side of the cell cords or tubules and never on the side toward the lumen. It is probable that this secretion finds its way into the numerous capillaries along the periphery. In the formation of two secretions, therefore, the hypophysis resembles the thyreoid in some forms, and also the hypophysis in some mammals.

2. *Histogenesis of the hypophysis*

In 7.5 mm. embryos the walls are formed of a single layer of cells of a large cuboidal type. The cytoplasm is slightly granular and somewhat acidophilic. The nuclei are large, somewhat oval, placed near the basement membrane, and contain considerable chromatin network. Usually several nucleoli, although sometimes only one, are found near the nuclear membrane (fig. 31). Very soon the walls of the hypophysis are composed of several layers of cells. In a 13 mm. embryo the large nucleoli are no longer so prominent and, as a rule, only smaller pseudonucleoli, as observed by Sterzi, are to be seen.

a. Anterior lobe. In a 21 mm. embryo, very little of the floor of the anterior lobe is as yet present. The walls are composed of two layers of columnar cells which have a thin outer and a thick inner, slightly granular cytoplasmic zone. The large and elongately oval nuclei contain a dense chromatin network, especially in the anterior end (fig. 32). Sterzi has observed that there are some elongated nuclei in the roof of the hypophysis in which the long axes are at right angles to the surfaces, and

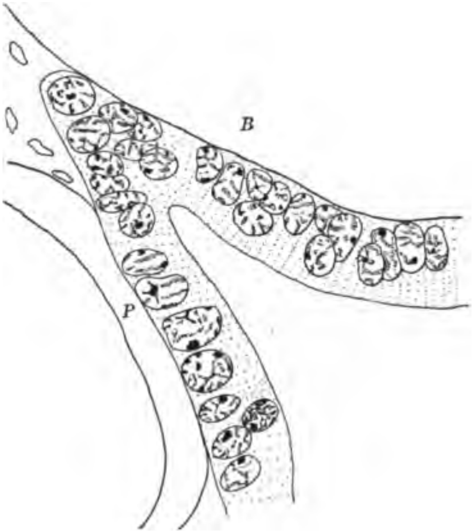


Fig. 31 Transverse section of a portion of the hypophyseal anlage of a 7.5 mm. embryo. (H.E.C. 1503). × 500. *B*, region of brain; *P*, premandibular somite.

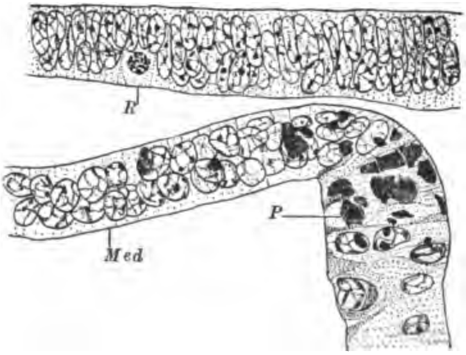


Fig. 32 Sagittal section of the roof of the anterior lobe in a 21 mm. embryo. (H.E.C. 1493). × 450. *R*, roof of anterior lobe; *Med*, medial connection of inferior lobes; *P*, pigment granules.

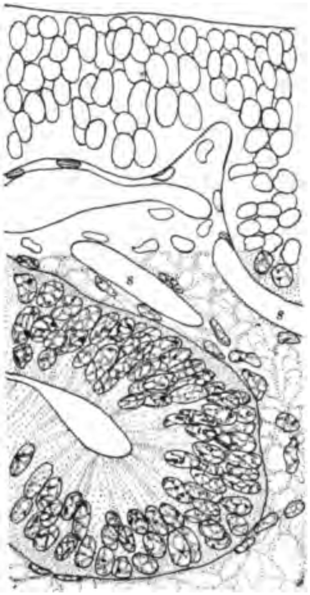


Fig. 33 Sagittal section of the floor of the anterior end and of one of the tubules of the anterior lobe of a pup. × 400.

between these are spherical shaped nuclei. He has described these in the roof of the superior lobe. These become more evident in 40 and 50 mm. embryos. In an 86 mm. embryo, the nuclei of the roof are to some extent irregular in arrangement. The cytoplasm takes orange G quite readily and hence is somewhat acidophilic. In the pup, the floor is well formed and is now the thicker wall of the anterior lobe. As shown in figure 33, it is composed of four or five layers of nuclei. The oval nuclei are crowded close to the inner free surface. There is, however, a narrow outer cytoplasmic zone which is very granular. The character of the cells of the glandular outgrowths is shown in figure 33. Here, too, there is a narrower outer rim of granular cytoplasm. A wider inner zone of non-granular cytoplasm bounds the lumina of the tubules. The nuclei, as in the floor, are oval. Usually two or three layers form the walls. The nuclei have a distinct chromatin network with occasional denser-staining chromatin masses resembling nucleoli.

b. Inferior lobes. The development of the inferior lobes as constrictions of the lateral walls of the early hypophyseal outpouching has been described above. The point where these lobes will grow together across the median line is indicated in figures 5 to 10. The character of the cells forming this part differs from the rest of the hypophyseal outpouching as early as the 21 mm. stage (fig. 32). The cytoplasm here stains less densely than that of the rest of the hypophysis. The nuclei are distinctly spherical in shape and have a very scant chromatin network. A part of this floor, immediately posterior to the hypophyseal stalk, contains a considerable amount of a granular yellowish pigment. Both Müller ('71) and Hoffmann ('96) have noted the pigment in the stalk of the hypophysis. The nuclei become more oval in older embryos. This is well shown in figure 34 which is a mid-sagittal section of the region which later forms the connection between the inferior sacs. The nuclei here are very irregularly placed. Extending caudally from the stalk are several nuclei flattened along the inner free surface. The cytoplasm stains very lightly. The pigment masses are numerous. Caudally there is a sudden transition to columnar cells of the kind found in the wall of the anterior lobe. The floor in this region is as just described until the 48 to 50 mm. stages when the inferior sacs are completely constricted from the anterior one. The pigment and the flattened nuclei are found only near the median line. Both are still present in a 41 mm. embryo.

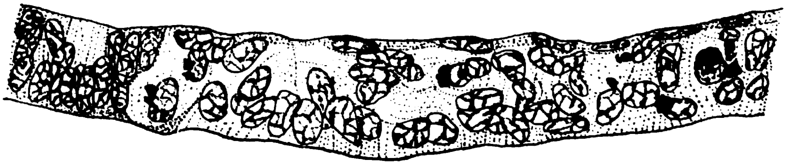


Fig. 34 Sagittal section of the floor of the hypophysis near the median plane, showing the part which connects the inferior lobes. (H.E.C. 362). × 400.

In the inferior sacs proper, however, which are formed at the lateral sides, the cells are similar to those of the floor of the anterior lobe. The outer, narrower zone of cytoplasm, as well

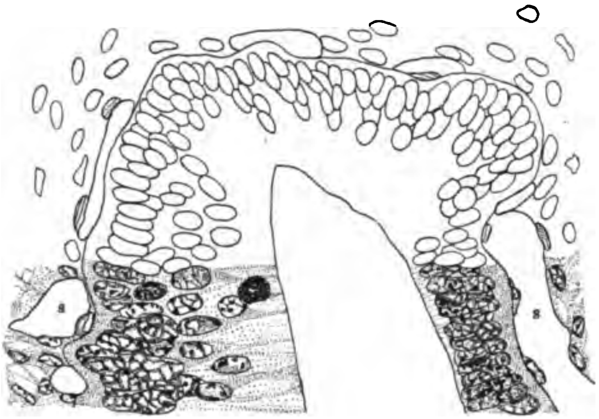
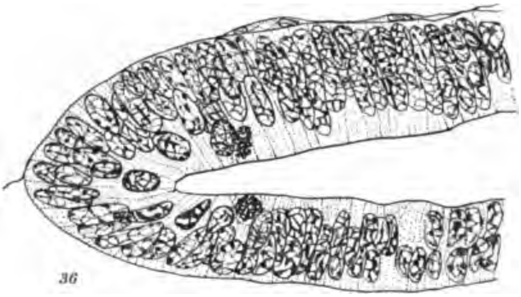


Fig. 35 Sagittal section of a portion of the inferior lobe of the hypophysis of a pup. × 400.

as the inner, wider one, stains lightly in a 50 mm. embryo. In an 86 mm. embryo the outer zone has fine granules. The cells are slightly acidophilic. The nuclei are elongately oval in outline as in the anterior lobe. In the pup many outpouchings indicate the beginning glandular development. The upper wall is three or four cell-layers thick (fig. 35). There is a wide, inner, clear-staining cytoplasmic zone. The outer narrower rim is slightly granular. The nuclei are oval in outline, as in the anterior lobe. Numerous densely staining chromatin masses are to be seen. The lower wall or floor is much thinner. It is composed of only one or two layers of cells and has a narrow, inner, cytoplasmic rim. This zone, as in the roof, stains very lightly and is non-granular. The outer rim is, however, quite granular. The nuclei are like those in the roof but contain a somewhat denser chromatin network.

c. *Superior lobe.* In a 21 mm. embryo the wall of the superior end of the hypophyseal anlage is thickest where the superior lobe later develops (fig. 36). The nuclei are large and oval and have a light chromatin network. In a 50 mm. embryo (fig. 37) the wall in this region is considerably thicker than in the 21 mm. embryo because of an increase in the number of cell layers. There



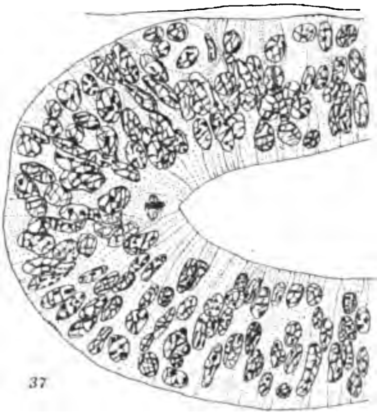
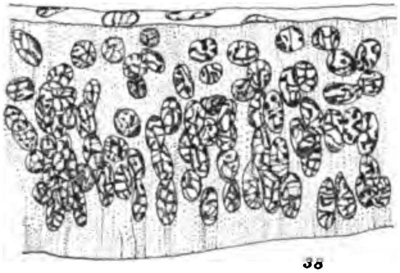


Fig. 36 Sagittal section of the superior end of the hypophysis of a 21 mm. embryo. (H.E.C. 1493). × 450.

Fig. 37 Sagittal section of the superior lobe of a 50 mm. embryo. (H.E.C. Series 444). × 450.

is an outer cytoplasmic zone which is non-granular and stains lightly. The nuclei are smaller than in the 21 mm. embryo and contain less chromatin. As Sterzi has stated, there are some spherical nuclei and some more slender oval nuclei, but no regular arrangement of these, such as Sterzi described, was observed. In a 95 mm. embryo, the roof of the superior lobe has increased in thickness (fig. 38). The nuclei are oval and contain a denser network of chromatin than is found in 50 mm. embryos. Along the periphery many of the nuclei are spherical. There is an inner zone of cytoplasm as in the 50 and 21 mm. stages, which is



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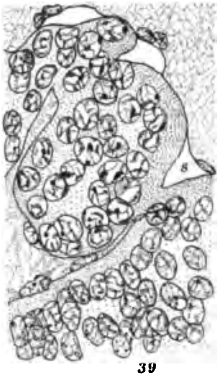

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Fig. 38 Transverse section of the superior lobe of a 95 mm. embryo. (H.E.C. Series 1882). × 450.

Fig. 39 Sagittal section of the superior lobe of the hypophysis of a pup showing one of the cell columns and a portion of the roof. × 400.

non-granular. Fewer mitotic figures are to be found at this time. In the pup the roof is a little thicker than in the preceding stage. Above the roof there are many cell columns which are outgrowths from the roof proper (fig. 39). The relations of cell columns and roof to each other are shown in figure 39. The roof has a narrow outer zone of granular cytoplasm. Its nuclei are oval and have a light chromatin network. The cell columns come into close contact with the overlying floor of the vascular sac (fig. 39). Numerous capillaries and a loose connective tissue fill the spaces between the cell columns and between the roof of the hypophysis and the floor of the vascular sac. The columns have an outer, granular cytoplasmic zone. The cells are acidophilic. The nuclei are spherical in outline, have a light chromatin network and usually one or two larger chromatin masses or nucleoli.

Only a brief statement can be given at this time concerning the development of the glandular columns of the superior lobe. In the region of the superior lobe in all embryos up to the 50 mm. stage, the nuclei are elongate-oval in outline and are arranged in two or three layers (fig. 36). In a 50 mm. embryo some of the nuclei at the periphery are spherical (fig. 37). It is possible that the elongate-oval nuclei in this region in the younger embryos are changed into spherical ones. In the pup there are numerous columns extending dorsally, which consist of a central group of spherical, light-staining nuclei and a peripheral zone of cytoplasm. Until some material between the 95 mm. embryos and the pup stage is studied, it must remain a question how these cell columns are formed. It is possible that the cells with spherical nuclei arrange themselves in groups and these groups then evaginate from the roof. The scarcity of these groups in the 95 mm. embryos and in all late embryonic stages argues against such a possibility, although Sterzi's observations on the presence of groups of spherical nuclei lying between masses of cells with oval nuclei should be taken into consideration. Numerous cyst-like outpouchings are present in some adults in the anterior part of the superior lobe, or, rather, between this and the roof of the caudal extremity of the anterior lobe. Some of these outpouchings show areas of cells similar to those forming the columns of the superior lobe, interspersed with areas of cells like those of the anterior lobe. The areas of cells resembling those of the superior lobe may form the entire wall or may lie on a basement of cells resembling those forming the anterior lobe which line the cavity. This would indicate that the regions of the anterior and superior lobes are not sharply separated, or, that the cells of this region which still resemble the embryonic condition change into cell columns of the superior lobe. This need not imply, however, that the cells of the anterior and inferior lobes are of a more embryonic type, although they may be more primitive phylogenetically.

3. Development of the interhypophyseal canal

The formation of the ridge connecting the inferior lobes on the dorsal (posterior) side of the hypophyseal anlage had been described. The character of the epithelium in this region in a 21 mm. embryo, as stated above, differs from that of other parts. A ridge is prominent in a 34 mm. embryo. In a 40 mm. embryo the groove on the inside of this ridge is marked (fig. 8). In a

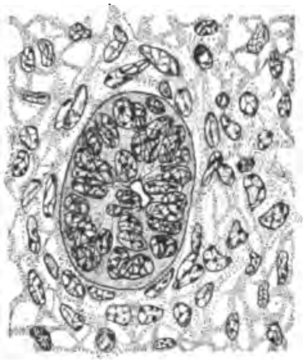
48 mm. embryo the ridge is very distinct and the connection of the lumen of this part with the lumen of the anterior lobe has become constricted. The constriction forms the narrowed connection of the inferior sacs to the anterior lobe. As previously stated, the growth of the furrows separating the inferior sacs from the lateral sides of the anterior lobe is well marked at this time. A sagittal section of the hypophysis of a 50 mm. embryo shows a short interhypophyseal canal (fig. 9). In a 95 mm. embryo the canal has lengthened considerably. The walls are composed of one or two layers of low columnar cells. In the pup the canal is longer than in the embryos but the diameter is about the same as in younger specimens. In the adult the canal has increased in length and diameter and is attached in the floor of the anterior lobe at or near its caudal end. Its other attachment is near the dorsal side of the connection between the inferior lobes. There are no tubular outgrowths from it such as Haller found in Mustelus. Table 4 shows the size of the canals at different stages. It will be seen that there is a continual increase in the length of the canal. The diameter in the older specimen is somewhat greater than in the 50 mm. embryo, although there is no great change. A distinct lumen is present. In the median line there is a well defined layer of connective tissue extending from the tip of the parachordal plate (fig. 9) forward to the floor of the anterior lobe of the hypophysis. This layer develops rapidly after the canal is formed and surrounds it. It is well defined in a 50 mm. embryo, but becomes thicker in the adult. This layer separates the inferior lobes from the rest of the hypophysis and

TABLE 4

Showing the size of the interhypophyseal canal

SIZE OF SPECIMEN	LENGTH OF CANAL IN MM.	DIAMETER OF CANAL IN MM.
Embryo of 50 mm.	0.13	0.042
95 mm.	0.36	0.048
Pup	0.44	0.048
Adult	0.68	0.080

makes their dissection difficult. The cells and fibers in it are arranged concentrically around the canal. In the adult, the layer of connective tissue is still prominent and extends the entire length of the canal.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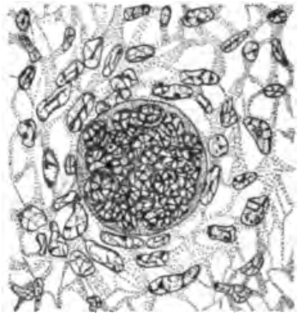
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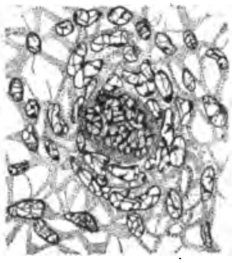
Fig. 40 Transverse section of the hypophyseal stalk of a 28 mm. embryo. (H.E.C. Series 1357). × 350.

Fig. 41 Transverse section of the hypophyseal stalk of a 33 mm. embryo. (H.E.C. Series 307). × 350.

4. Development of the hypophyseal stalk

The stalk connecting the hypophysis with the mouth is formed in 22 to 24 mm. embryos. It is present in one 21 mm. embryo which shows the anterior end constricting from the mouth. In these stages it is oval in cross-section, the lumen is very large, and its walls are formed of a layer of low columnar cells, the nuclei of which are somewhat elongated and contain considerable chromatin. In the posterior (superior) margin are found many yellow pigment granules, as has been described by Hoffmann ('86). These granules are found within the cell, sometimes apparently in the nucleus (fig. 32). As Hoffmann stated, they are first seen in the bucco-pharyngeal membrane, but later occur also in the wall of the stalk. Müller ('71) had described them in the stalk in Acanthias embryos of 30 mm. length.

In 28 mm. embryos the lumen in the stalk is small. The wall consists of a double layer of epithelial cells, the outer of which



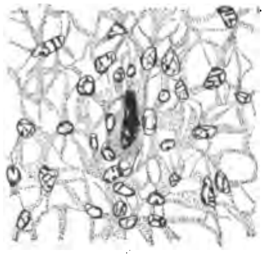


Fig. 42 Transverse section of the hypophyseal stalk of a 37 mm. embryo. (H.E.C. Series 363). × 350.

Fig. 43 Transverse section of the hypophyseal stalk of a 41.5 mm. embryo. (H.E.C. Series 369). × 350.

is low columnar in type (fig. 40); the inner is irregular and the nuclei are oval in outline but not regularly placed. Figure 40 is of a section through approximately the middle of the stalk. The lumen and stalk are larger where it connects with the hypophysis and with the mouth, both ends being funnel-shaped and joined by a narrower middle part. The connective tissue around the stalk is mesenchymal in character. Immediately around the stalk the cells are concentrically arranged. This character is more pronounced in later stages.

In a 33 mm. embryo the two ends of the stalk are funnel-shaped, as before, and contain a lumen, while the middle part of the stalk is made up of a mass of cells in which no lumen is present (fig. 41). The outer layer of cells, though still definite at the ends, is no longer so in the middle part. The nuclei are more spherical in shape and contain a denser chromatin network. The arrangement of the mesenchymal cells around the stalk is concentric.

In a 37 mm. embryo the stalk is greatly reduced in size. The nuclei are massed in the center and are surrounded by a densely staining cytoplasm (fig. 42). Some pigment is scattered throughout the stalk, as in all of the specimens. The concentric arrangement of connective tissue cells is more marked.

In some embryos 40 mm. in length no remnant of the stalk is found. In a 41.5 mm. embryo a small elongated densely staining stalk is present (fig. 43). What apparently is the remains of the nuclear mass is surrounded by a narrow densely-staining cytoplasmic rim. The chromatin network has disappeared, but larger masses of densely staining chromatin are to be seen. The concentric arrangement of the connective tissue cells is apparent, but not so marked as in the younger stages. In another specimen, 40 mm. in length, a strand of connective tissue, extending from a funnel-shaped mass of epithelial cells—continuous with the epithelial lining of the mouth—to the base of the hypophysis, indicates the position of the degenerated stalk (fig. 8). The hypophyseal attachment of the stalk is anterior to the groove connecting the inferior lobes. Soon after this time the cartilages at the base of the brain become continuous across the mid line.

I wish to thank Dr. R. E. Scammon for his many helpful suggestions throughout this work. Thanks are also due to Dr. R. J. Terry for his kindly interest during its completion.

SUMMARY

1. The terms 'anterior lobe,' 'inferior lobes' and 'superior lobe' have been used for the several parts of the hypophysis of Acanthias.

2. Rathke's pouch forms the posterior part of the anterior lobe. The later evagination of the ectoderm, anterior to this, forms the middle portion and the anterior extremity of the anterior lobe.

3. The inferior lobes develop from the lateral sides of the posterior extremity of the anterior lobe, i.e., from the lateral sides of Rathke's pouch.

4. The superior lobe develops from the caudal (superior) end of the hypophyseal anlage.

5. In the course of development the hypophysis shifts in position about 145 degrees, so that the upper wall becomes the floor and the ventral (anterior) surface the roof.

6. There is glandular growth from the roof of the superior lobe and the inferior lobes, as has been described by Sterzi and others, and from the floor of only the anterior and posterior extremity of the anterior lobe in all adults.

7. The cells of both anterior and inferior lobes are acidophilic in character.

8. The cell columns of the superior lobe are solid as Sterzi described them.

9. Frequently the anterior and inferior lobes stain more densely than does the superior lobe. In these cases, it is the nuclei which take the darker stain. In general the anterior and inferior lobes may be considered the chromophilic ones.

10. Spaces containing some colloid-like secretion are present in the superior lobe. A similar secretion is present in the lumina of the tubules of the anterior and inferior lobes and in the large main lumen.

ADDENDUM

After the completion of the present work a paper by M. W. Woerdeman: "Vergleichende Ontogenie der Hypophysis" appeared (Arch. f. mikr. Anat., Bd. 86). This investigator figured Rathke's pouch in an 8 mm. Torpedo embryo. In somewhat older embryos (12–15 mm.) the region where Rathke's pouch opens into the mouth evaginates and in still later embryos a region anterior to this is constricted from the mouth. The hypophysis then consists of a small Rathke's pouch somewhat constricted from an anterior (ventral) 'Mittelraum' and anterior to the latter, the 'Vorraum.' The middle division, in 20 mm. embryos, divides by a circular constriction into a dorsal and a ventral part. In this way the ventral sacs are formed. The hypophyseal stalk now opens into the ventral sacs, in which observation Woerdeman agrees with that of Gentes and Stendell. According to Woerdemann's comparison of the parts of the hypophysis with those described by Stendell, Rathke's pouch is homologous with the superior lobe (table 1, p. 400) and the 'Mittelraum' and 'Vorraum' are homologous with the anterior lobe. The ventral sacs and lateral lobuli which he described are probably homologous with the inferior lobes. In Squalus I have described Rathke's pouch, or the early anlage of the hypophysis as giving rise to the caudal extremity of the anterior lobe. A later evagination ventral to this gives rise to the middle portion and anterior extremity of the anterior lobe. The secondary evagination is early recognized, as the epithelium here is thickened (page 408). The opening from the mouth to the early hypophyseal anlage, or Rathke's pouch, secondarily comes to open in the later evagination (page 410) of which there is only one in Squalus. The inferior lobes and the superior lobe develop from the early hypophyseal anlage in Squalus, as has been described (pp. 410–11). The hypophyseal stalk is not constricted from the anterior lobe with the developing ventral lobes but remains connected with the caudal wall of the middle portion of the anterior lobe until it disappears.

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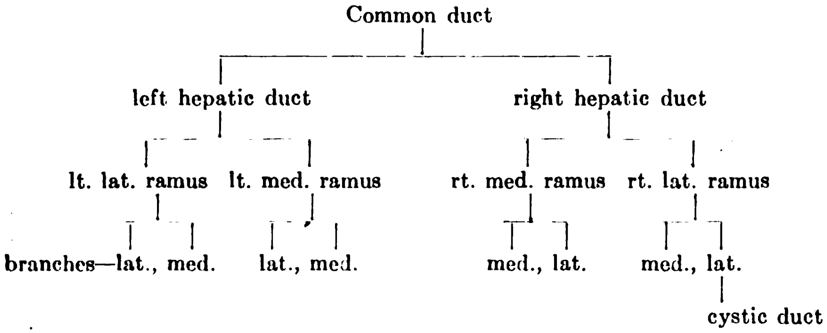
Reprinted from The Anatomical Record, Vol. 8, No. 2
February, 1914

Proceedings of the American Association of Anatomists, thirtieth session, at the University of Pennsylvania, Philadelphia, Pa. December 31, 1913.

49. *The development of the gall bladder and bile ducts in amblystoma.* E. A. **Baumgartner**, Institute of Anatomy, University of Minnesota.

Models of embryos 4.5 mm. long show that the liver anlage is a ventral, somewhat caudal projection of the gut lumen caudal to the heart. That this does not correspond to the caudal hepatic duct described in chick is shown by later stages. In models of embryos about 7 mm. long we see this early ventral outpouching has turned cranialward. In the ventral wall where the primitive common duct joins the gut lumen a median depression shows the earliest anlage of the gall bladder. In stages of about 9 mm. length the folds have become numerous and are the anlage of radicles of the main hepatic ducts. That they are not entirely formed by the tunneling in of blood vessels as has been described by Short, is shown by the fact that furrows are found in which no blood vessels are present. The early anterior, and laterally extending duct shows beginning constriction and elongation into right and left hepatic ducts. A little later the gall bladder has shifted to the right and is no longer widest transversely. The cystic duct also has shifted to the right, now being attached to the ventral side of the right hepatic duct. The shifting of the gall bladder is accompanied by a shifting of the entire caudal part of the liver due to the sinistral and ventral growth of the stomach and duodenum.

From graphic reconstructions of late embryos we see an arrangement of the biliary apparatus as shown in the following outline:



Occasionally the left medial and right medial rami join to form one duct which subdivides as a single stem. This is a secondary union and persists in the adult. Also in some cases the cystic duct was found to be one subdivision further removed, that is, it was attached to a radicle of the lateral branch of the right hepatic ramus.

In embryos between 10 and 12 mm. in length, division and growth of the early duct into right and left hepatic ducts and of these into rami takes place. A graphic reconstruction of a 13 mm. stage shows that this has taken place. The gall bladder here is now longest postero-anteriorly and the short cystic duct extends upward and to the left. A model of a 14 mm. embryo shows that the gall bladder has shifted more to the right and that its duct which is attached to the extreme anterior end extends somewhat upward and to the left.

A model of the biliary apparatus of a slightly older embryo shows greater development of the duct system. The gall bladder is longer anteroposteriorly, its duct is attached more caudally and is almost horizontal. In a 15 mm. embryo there is a short common duct, but the right and left hepatic ducts are longer. The right lateral shifting here is more marked. The cystic duct extends horizontally to the left. This stage marks the extreme caudal attachment of the cystic duct to the gall bladder.

At the 20 mm. stage the lateralward shifting is very marked. The right hepatic duct is now somewhat dorsal to the left. Here the right and left medial rami have united to form one duct. The gall bladder has increased very materially in size, particularly in its caudo-cranial diameter. The cystic duct has shifted toward the anterior end and now projects to the left and downward. The extreme of the lateralward shifting has been reached in a 35 mm. embryo. The right hepatic duct here is quite dorsal in position in relation to the left. Its lateral radicles are also more dorsal than its medial. The left lateral ramus has turned caudalward to supply hepatic radicles to the further caudal extending left lobe of the liver. The gall bladder has increased in its dorso-ventral diameter. Its duct is attached at the anterior end and extends toward the left and downward as before. In a 45 mm. embryo and larger ones the right and left hepatic ducts are again more nearly in a horizontal plane. The cystic duct is attached to the anterior medial end of the gall bladder.

Summary. (1) The ductus choledochus develops as the early anterior directed duct from the gut. (2) The right and left hepatic ducts develop as divisions of the ductus choledochus and by growth and division form the hepatic rami and branches. (3) The gall bladder begins as a median ventral outpouching of the early anterior forming liver anlage. It is first widest laterally and finally obliquely caudo-cranially. (4) There is an early right lateral shifting of the biliary apparatus. Along with this there is a constant shifting direction of the cystic duct in keeping with the dorsalward migration of the gall bladder. (5) The cystic duct is early closed off with the right hepatic and finally is attached to the lateral branch of the right lateral ramus.

Reprinted from The Anatomical Record, Vol. 8, No. 7
July, 1914

ON THE VASCULARIZATION OF THE SPINAL CORD OF THE PIG

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FIVE FIGURES

HISTORICAL

Until 1904 little work had been done upon the development of the blood-vessels of the spinal cord, except that of His ('86) who undertook to follow the growth of these vessels in human embryos. The observations of this author have been largely disputed by Sterzi ('04) and Evans ('09), the two writers who have done most of the work in this field.

By far the most comprehensive publication upon the development of the vessels of the spinal cord is that of Sterzi ('04). In this he discusses the development of the vessels in the five higher classes of vertebrates. As a type of the Mammalia he uses the sheep. He brings out the following points:

The blood-vessels first approach the cord at the ventro-lateral border and spread over the ventral surface, then over the lateral, and finally over the dorsal surface. Each vertebro-medullary artery as it approaches the cord divides into a ventral and a dorsal ramus, the ventral and dorsal radical arteries. The ventral radicals from either side halt at the lateral edges of the floor-plate, and each divides into a cranial and a caudal branch. These anastomose with those of adjacent segments and form two longitudinal arteries on the ventral surface of the cord, the "tractus arteriosus primitivus." Later they send out medial rami and through these become connected. Still later, alternate parts of the two tracts degenerate while other parts continue to develop. These enlarged segments are joined together through their medial rami and form a single ventral artery which Sterzi terms the tractus arteriosus ventralis, and which is the anterior spinal artery of most authors. From the primitive tract, dorsal rami enter the substance of the cord. Each dorsal ramus forms a loop and gives rise to a vein, which courses ventrally and enters the primitive sulcus. Later other vessels extend into the cord from the lateral, and still later from the dorsal surface.

The dorsal radical arteries, where they divide, form many small longitudinal capillaries just ventral to the points of emergence of the dorsal nerve roots. From these capillaries there is formed later a longitudinal artery on either side of the cord, in this plane (tractus arteriosus lateralis). The vessels entering the cord are first solid and later become hollow.

Evans ('09) shows by a series of injected pigs the early development of anterior spinal artery In his figures the mid-ventral surface of the cord is shown to be free from vessels until the embryos are 8.5 mm. in length, and the mid-posterior surface until after the pigs are between 8 and 10.5 mm. in length. He does not take up the later stages.

MATERIAL AND METHODS

For a study of this nature, injected embryos are indispensable, and they are best injected while living, with warm india ink diluted one-half with weak ammonia water. It is preferable to inject through the umbilical artery rather than through the umbilical vein, because the arteries are less readily ruptured and because the route between them and the vessels of the spinal cord is much more direct.

Embryos used for thin serial sections are better if they are congested instead of being injected. This congestion is accomplished as follows: The umbilical cord is tied while the embryo is yet living, thus causing an increase in the blood pressure in the aorta. One of the most direct outlets for this increased pressure is the system of segmental arteries, and through these the bloodvessels in and around the spinal cord soon become engorged. When this condition is reached, as evidenced by the increased redness of the dorsal region of the embryo, the live embryo is dropped into a fixing fluid which penetrates rapidly so that the capillaries are fixed before they collapse. Bouin's picro-formo-acetic mixture serves this purpose very well. Embryos treated in this manner show the smaller vessels much more plainly than those fixed in the usual way.

Small injected embryos which have been cleared in oil may be dissected under the binocular microscope and all the external vessels of the cord demonstrated, or they may be sectioned in celloidin to show the internal vessels.

From pigs larger than 25 or 30 mm. the cord with its membranes may be dissected out and embedded in celloidin and cleared, or for temporary preparations may be cleared directly.

Serial sections of the cleared embryo or spinal cord can be kept permanently between two pieces of paper soaked in oil, or can be transferred to slides and mounted in damar gum. To make slide preparations, the sections are cut in oil and placed on a piece of thin paper in the same order they are to have on the slide. Another piece of oiled paper is laid down upon them and the whole inverted. The first paper is now peeled off and the other paper holding the sections is inverted upon the slide. This paper is then peeled off, leaving the sections on the slide in their proper order. They may then be washed carefully with xylol, and covered.

There are several advantages in a study of this kind, in section ing celloidin-embedded embryos free-hand in oil. The cord can be turned so that sections may be cut through any plane. Sectioning is done more rapidly than with a microtome, and much time is saved. It is easy to make transverse, sagittal and frontal sections from any region of the same cord. The block can be examined with a lens, during the sectioning, and any particular vessel or vessels included in a section. Also, sections may be made of different shapes.

BLOOD-VESSELS OF THE NEAR FULL-TERM FETUS

In order to determine as near as possible the arrangement of the blood-vessels of the spinal cord in the adult condition, a number of fetal pigs, near full-term, were injected and their spinal cords dissected out for study. Although these showed some variation in the blood-vessels, as is to be expected, a general plan was to be observed. The following description is of the typical condition found in the blood-vessels in and around the spinal cord of pigs of about 240 mm. in length:

The terminology used by Kadyi ('89) for the blood-vessels of the adult human cord will be referred to frequently.

On the surface of the cord at this stage are four main longitudinal arterial systems which are located: one, median on the ventral surface; one, on each dorso-lateral surface; and one median on the dorsal surface.

The vertebro-medullary branches of the dorsal segmental arteries approach the cord laterally and each divides into a dorsal and a ventral branch termed the posterior and ventral radical arteries respectively (figs. 1 and 2). The latter reach the cord along the cranial surface of the spinal nerves and ganglia, and, on the cord, run cranially. They may be equally distributed to the two sides of the cord and to the different regions of it, or some regions may have more than others. They average eighteen in number. The ventral and dorsal radical arteries have lost their connection with the vertebro-medullary artery in places and extend between the artery on the ventral surface of the cord and one of those on the dorso-lateral surfaces.

Soon after reaching the cord each ventral radical artery branches, giving off one ramus which courses cranially, and another which extends caudally. These divisions anastomose with those of neighboring ventral radicals, on the same or opposite side

Fig. 1[1] Ventral surface of the middle thoracic region of the spinal cord of a fetal pig, 240 mm. in length. *A.S.A.*, anterior spinal artery; *A.R.V.*, ventral radical artery; *T.A.P.*, accessory anterior spinal artery (remains of primitive arterial tract); *V.R.V.*, ventral radical vein; *V.S.A.*, anterior spinal vein; *C.P.*, capillary plexus. × 6.

Fig. 2 Dorsal surface of the same part of the cord shown in figure 1. *A.M.D.*, median dorsal artery; *A.P.L.*, dorso-lateral artery; *A.R.D.*, dorsal radical artery; *V.M.D.*, median dorsal vein; *V.P.L.*, dorso-lateral vein and plexus; *V.R.D.*, dorsal radical vein; *V.L.*, dorso-lateral venous plexus. × 10.

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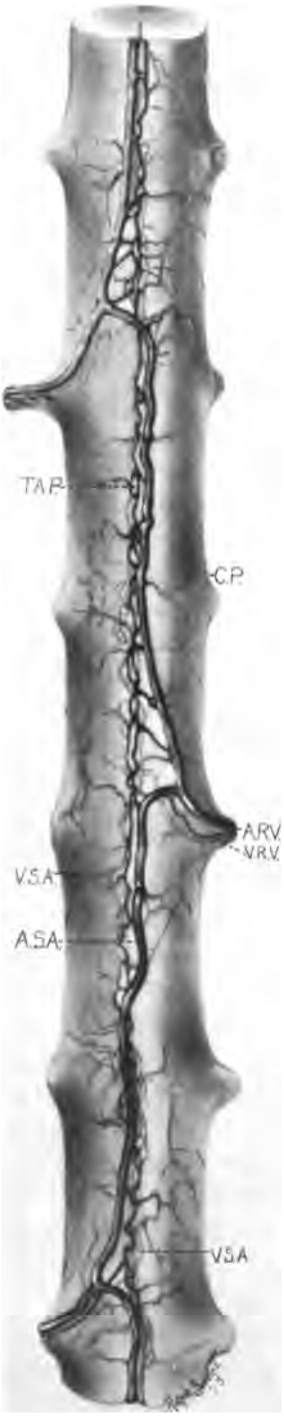
[1] The figures in this paper, except numbers 3 and 4, were drawn with a camera lucida. The size of the embryos is the greatest length, as measured in 75 per cent alcohol.



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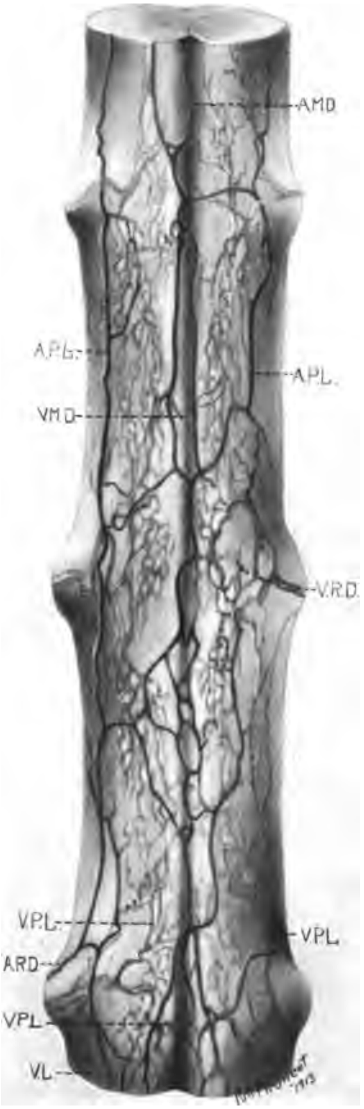

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of the cord, and form in this way the anterior spinal artery, which lies in or near the median ventral line. Occasionally a radical artery, instead of dividing, goes across the cord and joins the cranial or caudal ramus of the one on the opposite side. The anterior spinal artery has a winding course, bending laterally to meet the vessels which form it, and making many smaller irregular bends to one side or the other. In some places it may lie to one side of the mid-line for several segments and where this occurs there are found numerous longitudinal rami of the anterior spinal artery, or the cranial and caudal divisions of the ventral radicals. These may be called accessory anterior spinal arteries, and are sometimes present even where the anterior spinal artery lies in the mid-line. Here they are located between this vessel and cord, at the lip of the ventral median fissure. They are the remnants of the "tractus arteriosus primitivus" of Sterzi.

The dorsal radicular rami of the vertebro-medullary arteries are much more numerous than the ventral ones. They course dorsally along the cord and in a slightly cranial direction, to a plane just ventral to the emergence of the dorsal roots of the spinal nerves, where they divide into two rami, one extending cranially and one caudally. Each of these rami anastomoses with the one of the adjacent segment, and thus there is formed on either side an irregular longitudinal vessel, the dorso-lateral artery (fig. 2; tractus arteriosus postero-lateralis of Kadyi). From this artery recurrent rami supply the dorsal nerve roots and spinal ganglia, and the lateral surface of the cord. Other rami, two or three in each segment, and much larger than the above, run dorsally and by longitudinal anastomoses with each other, and with similar rami from the opposite side, form an artery in the mid-dorsal line which may be termed the median dorsal artery.

Very small rami from the dorso-lateral arteries run ventrally along the cord and unite with others from the anterior spinal artery, forming a plexus on the ventral and lateral sides. These ventral rami of the dorso-laterals anastomose freely in a longitudinal direction and form one or more small longitudinal arteries between the ventral and dorsal nerve roots in some parts of the cord. These are the tracti arteriosi laterales, and ventro-laterales, of Kadyi. Still other small rami from the dorso-lateral and median dorsal arteries form a capillary plexus on the dorsal and dorso-lateral surfaces of the cord. In a few places along the cord the dorso-lateral arteries are double, one division lying dorsal to the dorsal nerve roots, and corresponding perhaps to the "tractus arteriosus posterior" of Kadyi.

The median dorsal artery is a very irregular longitudinal vessel formed by the dorsal rami of the dorso-lateral arteries, as described above. In places it is double or may show a longitudinal capillary arrangement. Many of its lateral rami anastomose longitudinally forming small arteries parallel with the median dorsal artery (fig. 2). This is also true of the dorsal rami of the dorso-laterals. By the anastomoses of the rami of the various arteries just described, the entire cord is surrounded by an arterial vascular system, and from all parts of this network smaller arteries penetrate its substance.

The veins of the spinal cord are in three principal longitudinal systems, and other smaller ones. Of the three, two are dorsal and one ventral. All three show evidence of their capillary origin. The anterior spinal vein is the smallest of the three (fig. 1). It lies between the cord and the arteries, in the median ventral line. It is larger than the accessory anterior spinal arteries, but never attains the size of the anterior spinal artery proper. It is very irregular and in some regions is entirely replaced by a narrow network of capillaries.

On either side of the median ventral sulcus, the cord is covered with large venules, some of which lie between the cord and the arteries, and some of which are external to the latter. They are often two or three times as large as the arterioles to which they correspond. They anastomose freely with the anterior spinal vein and empty laterally into the ventral radical veins which are in close relation with the ventral nerve roots and ventral radical arteries, but which are much more numerous than the latter, one being present on nearly every nerve root. They drain blood also from the lateral surface of the cord. Their ventral and dorsal rami often form short, small, longitudinal veins by anastomoses, some of which in other animals have been named, antero-lateral, etc. The blood from the anterior spinal veins and venous capillaries of the general ventral surface form, in places, transverse channels which are perhaps large enough to be called veins.

On either side of the dorsal surface of the cord there extends longitudinally a large irregular vein, the dorso-lateral, about half way between the artery of the same name, and the median dorsal artery. Some parts of these vessels and their rami, like the ventral venous capillaries, lie external to the arteries and some internal to them. They are the largest vessels on the cord with the exception of the anterior spinal artery (compare figs. 1 and 2). Dorsally these veins are united through large capillaries, and blood leaving the cord in the median line may flow either to the right or left. Half way between two consecutive nerve roots the dorsolateral veins usually break up into many divisions so that each may be seen to drain blood from adjacent halves of two segments. Laterally they empty through one or more divisions into the large dorsal radical veins, one of which lies upon each dorsal nerve root (fig. 2).

A fourth longitudinal venous system, smaller than the three described, lies in the median dorsal sulcus. It resembles the dorso-lateral veins except that it is more irregular, and in places it may be entirely lacking. Its lateral rami empty in the dorsal venous capillary plexus or directly through larger vessels into the dorso-lateral veins. It may be termed median dorsal venous system.

Some of the venous capillaries of the lateral surface drain into the dorso radical veins, some into the dorso-lateral veins, and some into ventro radical veins, and all these vessels together with the anastomoses of the veins on the ventral and dorsal surfaces already mentioned above, completely surround the cord with a venous system, corresponding to the system described for the arteries.

Of the arteries entering the cord, the largest are those in the ventral fissure, the ventral central arteries, which form two nearly parallel rows, but which are not paired. They arise from the anterior spinal arteries, or the accessory anterior spinal arteries. They show evidence of the capillary origin in longitudinal anastomoses found between vessels of the same side. These anastomoses are numerous in the fissure, particularly near the vessels from which the ventral central arteries arise.

The ventral central arteries vary considerably in size, some being as large as the vessels they arise from and others much smaller (fig. 3). The course of the smaller vessels is usually more irregular than that of the larger. They pierce the substance of the cord at different levels, some entering near their origin and others extending some distance into the fissure. Their general course is dorso-lateral, but those entering near the mouth of the fissure may bend very sharply to the side and enter the ventral horn of the gray substance. The others course more dorsally nearly to the level of the central canal where they make a decided lateral bend, and divide into two or more rami, although sometimes they give off rami more ventrally than this (figs. 3 and 4). The principal divisions of these arteries extend in a longitudinal plane, and anastomose with similar rami of adjacent vessels. They also give off smaller arteries and capillaries which ramify through the gray matter in all directions, helping to form a dense plexus. The longitudinal arteries tend to form loops after they have coursed in one direction for a short distance, as they do in young embryos (fig. 5). One artery may form several such loops, producing as many longitudinal vessels, each succeeding vessel lying dorsal or lateral to the last, and of a lesser caliber. These smaller longitudinal vessels anastomose with each other ventro-dorsally and laterally by rami which usually leave them at right angles, and also anastomose with rami from vessels other than the ventral central arteries, as will be described later.

Besides the rami of the central arteries just described, other rami extend farther laterally into the gray substance before branching. Some of these, instead of forming longitudinal vessels, form small irregular ones which ramify through the gray matter in all directions, anastomosing with similar vessels from other arteries in this region and forming a dense capillary plexus in the ventral and dorsal horns.

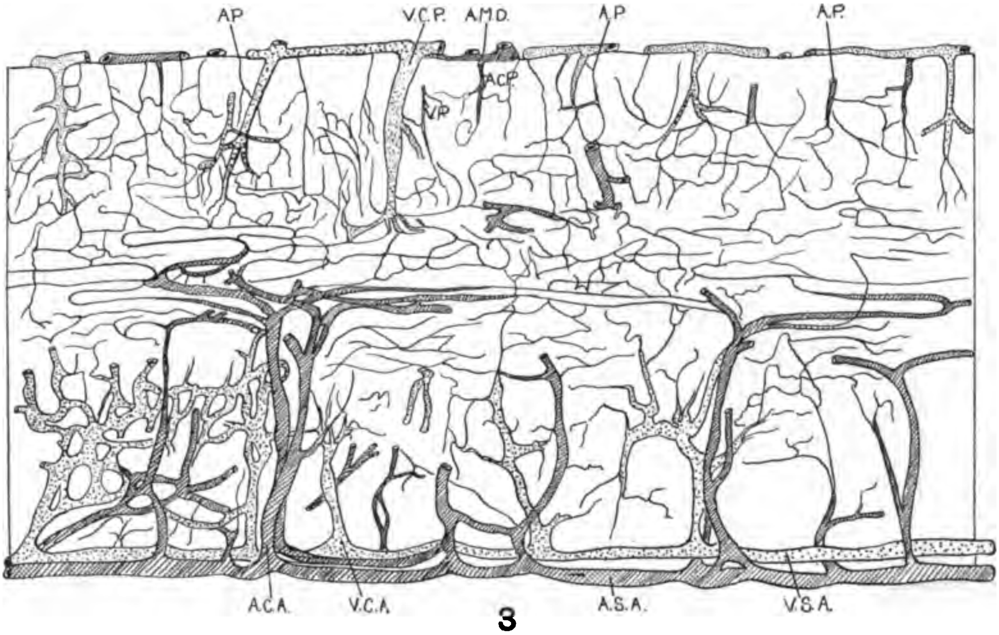
Other arteries, smaller than the ventral central arteries, enter the cord from the dorsal median sulcus and course ventrally and laterally to the dorsal horns of the gray substance. Here they form still smaller vessels resembling to some extent those formed by the ventral central arteries, but most of their rami are short and do not extend longitudinally. These may be called the dorsal central arteries, but they are more similar to the peripheral arteries from other surfaces of the cord, than to the ventral centrals, and perhaps should be called dorsal peripheral arteries. They give off many small lateral rami in the white substance and in the outer part of the gray substance.

In addition to these vessels, other small arteries enter the cord from all sides, from the arteries and arterioles which surround it. These are the peripheral arteries referred to above. They are very numerous and in a single thick cross section as many as fifty or sixty of them may be counted. They give off short rami in the white layer of the cord and extend into the gray layer. These rami branch and anastomose and form a loose capillary network. The vessels entering the gray substance enter into the longitudinal plexus already described and give off lateral rami which branch freely and anastomose.

The longitudinal vessels which arise from the ventral central arteries are quite large, but the other vessels formed from these trunks, and those formed from the dorsal central and peripheral arteries, are much smaller. A very thick section presents a picture of an inner core of longitudinal vessels with other vessels extending into it at right angles from all points on the periphery of the cord (figs. 3 and 4).

Fig. 3 Sagittal section from the lower thoracic region of the spinal cord of a 240 mm. fetal pig. *A.S.A.*, anterior spinal artery; *A.C.A.*, ventral central artery; *A.P.*, peripheral artery; *A.C.P.*, dorsal central artery; *V.S.A.*, anterior spinal vein; *V.C.A.*, ventral central vein; *V.C.P.*, dorsal central vein; *V.P.*, peripheral vein. × 35.

Fig. 4 Transverse section through the lower thoracic region of the spinal cord of a 240 mm. fetal pig. *A.S.A.*, anterior spinal artery; *A.P.*, peripheral artery; *A.*, artery; *A.C.A.*, ventral central artery; *S.A.S.A.*, accessory anterior spinal artery; *A.P.L.*, dorso-lateral artery; *A.M.D.*, median dorsal artery; *A.S.V.*, anterior spinal vein; *V.*, vein; *V.P.*, peripheral vein; *V.P.L.*, dorso-lateral vein; *V.M.D.*, median dorsal vein. × 35.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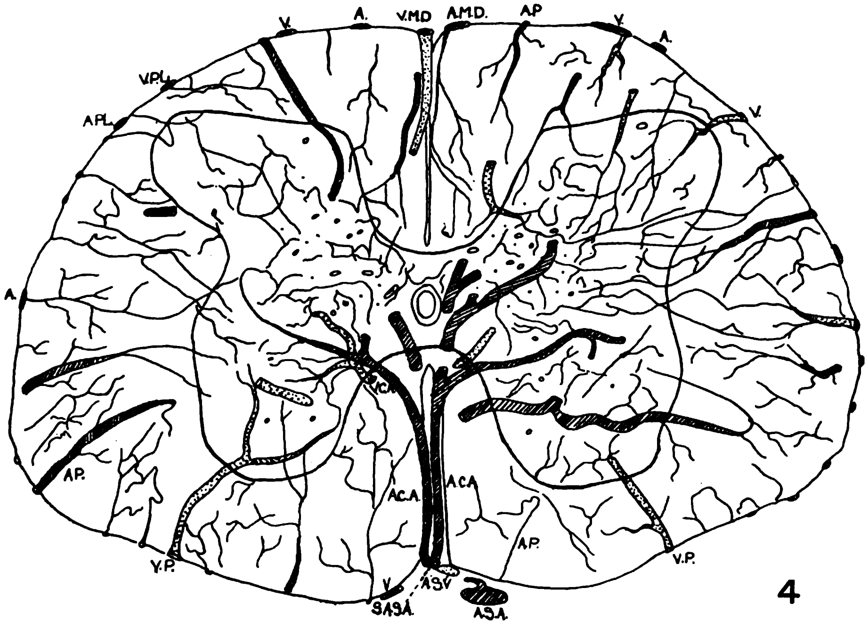
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4

DEVELOPMENT OF THE BLOOD-VESSELS

The early development of the anterior spinal artery has been described by Evans ('09) and Sterzi ('04).

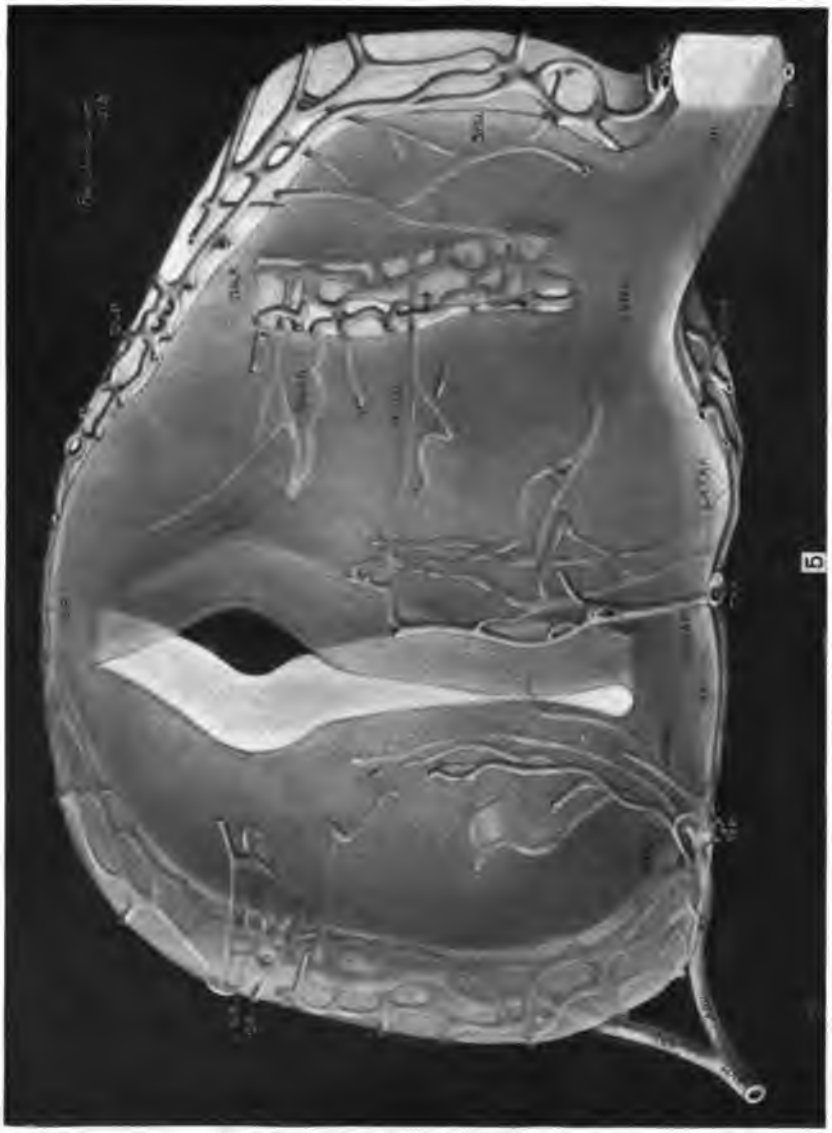
Some pig embryos of 12 mm. show a fairly well developed anterior spinal artery, while in others of 14 or 15 mm. it is just beginning to form. Although, after this vessel is once formed, it does not undergo marked changes, there is some modification. For example, the ventral radical arteries meet it at right angles or nearly so, until the embryo is 40 or 45 mm. in length. Thereafter, the growth of the cord and the fixed position of the radicals seem to cause the artery to be pulled laterally by the radicals, and a gradually decreasing angle is formed at the points where the radicals meet it (fig. 1).

As the embryo grows, the number of radical arteries continues to decrease even after the anterior spinal artery is well formed. This seems to be true until the embryo reaches the length of about 100 mm.

Some of the arterial capillaries on the ventral surface of the spinal cord are continuous with the anterior spinal artery directly, or indirectly through remains of the tractus arteriosus primitivus, and others are continuous with the capillaries of the lateral surfaces of the cord.

A dorso-lateral artery is formed in the capillary plexus on each of the lateral surfaces of the cord, just ventral to the point of emergence of the dorsal nerve roots. The dorsal radical arteries branch in this region and give off dorsal and lateral rami, which are continuous with the lateral capillaries just mentioned. A very irregular longitudinal vessel develops where certain of these

Fig. 5 Transverse section through the mid thoracic region of the spinal cord of an 11 mm. pig embryo. *T.A.P.*, primitive arterial tract; *R.D.T.A.P.*, *R.L. T.A.P.*, *R.M.*, dorsal, lateral, and medial rami of the primitive arterial tract; *A.V.M.*, vertebro-medullary artery; *A.R.V.*, *A.R.D.*, ventral and dorsal radical arteries; *C.R.*, *Cr.R.*, *D.R.*, *V.R.*, caudal, cranial, dorsal and ventral rami of the dorsal radical artery; *D.P.*, *D.L.P.*, dorsal and dorso-lateral capillary plexuses; *D.L.C.G.*, *V.L.C.G.*, dorso-lateral and ventro-lateral groups of peripheral capillaries; *V.L.V.P.*, ventro-lateral venous plexus; *V.R.V.*, *V.R.D.*, ventral and dorsal radical veins; *S.P.G.*, spinal ganglion; *V.N.R.*, *D.N.R.*, ventral and dorsal nerve roots; *S.N.*, spinal nerve. × 200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capillaries increase in size, perhaps on account of the increased pressure from the dorsal radical arteries. This longitudinal vessel is indicated in embryos of 12 mm. and is quite strongly developed in embryos of 15 to 18 mm. In these stages it seems to dip ventrally to meet the approaching radicals, as pointed out by Sterzi for the sheep ('04). As the embryo grows, this dorso-lateral artery becomes more and more regular. It is still somewhat irregular in embryos of 60 mm. but quite regular in those of 75 mm. The dorso-lateral artery never attains the size of the anterior spinal nor is it ever so regular in its course. In places it may develop as two or more vessels, but these are always smaller than the single artery. The dorso-lateral arteries are each continuous with the capillaries of half the cord in the early stages, but as the cord increases in size they supply directly only the dorso-lateral surface.

The capillary network on the lateral surface of the cord is at first continuous with that extending through the mesenchyma of this region as far, laterally and dorsally, as the myotomes and body wall respectively. In later stages when the membranes of the cord begin to develop, the connections between the vessels of the cord and those in the mesenchyma around it are lost.

The median dorsal artery is the last of all the vessels on the cord to develop. In pigs of 30 mm. it is still very irregular and indefinite, and is entirely lacking in places, although the vessels which go to form it, the dorsal rami of the dorso-lateral arteries, may be seen in embryos of 20 mm. In pigs of 45 mm. it is quite definite, lying in or near the mid line of the dorsal surface, as described above for the 240 mm. embryo It never becomes very regular, and in pigs of 100 mm. it resembles the condition seen in the pig of 240 mm. It is continuous with the arterial capillaries of the dorsal surface of the cord and with the dorso-lateral arteries.

In addition to these main arterial trunks there develop on various parts of the cord, especially on the lateral surfaces, short longitudinal arteries. These are never large or regular. They have been described in connection with adult human cord under the terms "tractus arteriosus; ventro-lateralis, posterioris, and lateralis" (Kadyi). Of these, the "tractus arteriosus posterior" is the most prominent and corresponds to the description in this paper of parts of the dorso-lateral artery, where it sometimes has two divisions, one of which runs dorsal to the dorsal nerve roots and the other ventral to them. The dorsal divisions are evidently the same as this 'tractus.'

The veins on the cord develop in much the same way as do the arteries. The ventro-lateral surface of the cord in very young embryos is covered with capillaries, and these are continuous laterally with the capillaries in the mesenchyma round the neural tube. Medially they become continuous with the lateral rami of the primitive arterial tract. When this tract becomes separated from the cord by the ingrowth of mesenchyma, these capillaries send medial outgrowths between the tract and the cord, as seen in embryos of 12 to 15 mm. Dorsally they grow along the cord and spread over the dorso-lateral surface (pigs of 6.2 mm.) and later over the dorsal surface (pigs of 7.5 mm.). Laterally they spread over the ganglia.

From the ventral surface, the blood draining away through the capillaries soon establishes segmental vessels, the ventral radical veins, which course laterally along the nerve roots. Each radical vein on one side drains adjacent halves of two segments. These receive blood from the capillaries of the ventral, lateral, and ventro-lateral surfaces. Lying in the ventral median fissure in young embryos, small longitudinal veins may be seen in different regions of the cord, and in embryos of 25 to 30 mm. a fairly definite longitudinal vessel may be found here. This vessel in still older embryos becomes a more definite trunk and may be called the anterior spinal vein. It never attains the size of the anterior spinal artery. Laterally it drains into the ventral radical veins.

Some of the ventral and lateral capillaries of the younger embryos, early become differentiated into veins. This is especially true of the dorsal vessels. From these, some of the blood drains laterally out through vessels in the mesenchyma to the myotomes. A pig of 6.2 mm. shows three planes in which this occurs, one on a level with the dorsal surface, one just above the level of the ventral surface, and one about half way between the other two. At the myotomes the blood drains ventrally into the intersegmental veins. Some of the capillaries of the lowest of these three planes, which drain the blood from the lateral surface of the cord and from the ganglia, soon become large and are called the vertebro-medullary veins, one pair of which is formed for each segment. In older embryos they course along the spinal nerves with the vertebro-medullary arteries. They receive the blood from the ventral and dorsal radical veins. The former have been described. The latter develop along the sides of the ganglia in the capillaries already mentioned. At first they carry only a part of the blood from the dorsal surface of the cord, but later (pigs of 25 mm.) they carry practically all of it. They are more numerous than the corresponding ventral radicals, and are found in every segment.

The venous capillaries of the dorsal-lateral surface on either side draining toward the nerve roots early establish longitudinal veins. These are only about half as long as a segment of the cord. Figure 5 of an 11 mm. pig, shows an indifferent plexus on this surface, but in 15 to 17 mm. embryos, fairly definite vessels may be seen. These become more and more regular as the animal develops, and as embryos of 50 to 60 mm. show, they form a venous system on either side of the cord just dorsal to the dorsal nerve roots, much like that described for the 240 mm. stage. These systems constitute the dorso-lateral veins (fig. 2).

The first blood vessels entering the cord grow in as capillaries from the ventral surface. Sterzi ('04) reports vessels in the cord of a sheep of 5.5 mm., but they were not apparent in the cord of pig embryos of less than 7.5 mm. These vessels are the dorsal rami of the primitive arterial tracts, of the lateral rami of these tracts, and of the other capillaries near the median line. They are the first indications of the central arteries and veins. They form two nearly parallel rows, one on either side of the ependymal layer, or some of them may lie in this layer. They grow dorsally about half way to the dorsal surface of the cord. They exhibit numerous longitudinal anastomoses and form a plexus along the ateral side of the ependymal layer in each half of the cord. These are true capillaries at first, but soon differentiate into arteries and veins.

Those coming directly from the primitive arterial tracts all become arteries, while those coming from the vessels lateral to the tract may become either veins or arteries.

In embryos of 9.5 mm. another group of capillaries may be seen to have entered the cord. These come from the lateral surface, extending medially nearly to the central canal. Later they anastomose ventro-dorsally and longitudinally, among themselves and with the vascular sprouts from the ventral surface.

The vessels in the cord of a pig of 11 mm. present the following characteristics, as shown in figure 5. Rami from the primitive arterial tract may anastomose with those from the ventral capillaries. Neighboring vessels of the same kind anastomose freely and give off lateral rami into the anlagen of the ventral horns of gray substance. These rami branch and anastomose with each other and form loops which anastomose with the central vessels from which they arise, or with neighboring vessels. In a plane just above the anlagen of the ventral horns each of the central vessels ends blindly, or divides into a caudal and a cranial ramus, which anastomose with adjacent similar rami and form irregular longitudinal vessels. By other anastomoses among the central vessels, a longitudinal plexus is formed, which covers very completely the lower half of the lateral side of the ependymal layer.

A comparison of figures 3 and 5 shows how closely the form and arrangement of these capillaries corresponds to that of the future central arteries and veins. Besides these main capillaries two smaller lateral groups are present at this stage. These may be called the ventro- and dorso-lateral groups, and later form peripheral arteries and veins. Both groups enter the cord from the capillaries on the lateral surface between the dorsal and ventral nerve roots. The ventro-lateral group enters at the level of the dorsal extremities of the central vessels, and courses medially and anastomoses with them. Occasionally the ventro-lateral group gives off rami which extend into the anlagen of the ventral horns. The capillaries of the dorso-lateral group are confined to the dorsal two-fifths of the cord, and although they anastomose with each other at this stage, they do not anastomose with the central or ventro-lateral capillaries. They course medially and dorsally along the ependyma, ending blindly or forming loops, but do not reach the dorsal surface.

As development proceeds, the lateral groups of capillaries shown in figure 5 spread dorsally and ventrally and capillaries enter the cord from the periphery. With the exception of the above-mentioned dorso-lateral group of capillaries, all the vessels entering the sides of the cord grow toward a common center, namely, an area on the lateral border of the ependyma about half way between the dorsal and ventral surfaces. The dorso-lateral group of capillaries which are shown in the same figure send rami toward this center after the embryo attains the length of 14 mm.

The vessels from the dorsal surface grow ventrally along the ependyma and unite with the dorsal rami of the primitive arterial tract. This union continues the plexus on the lower part of the ependyma dorsally so that the ependyma except below the floor-plate and above the roof-plate, is entirely surrounded by a capillary plexus. A thick transverse section of the cord of an embryo of 25 mm. shows this plexus with numerous vessels extending from it laterally at right angles. These lateral vessels are joined together by dorso-ventral rami. This picture is characteristic of the cord until the embryo reaches the length of 30 or 35 mm. when it is changed by other peripheral vessels meeting the ependymal plexus obliquely and by the branching of the vessels in the anlage of the gray substance.

By this time the central arteries from both the ventral surface (ventral central arteries) and from the dorsal surface (dorsal central or dorsal peripheral arteries) have become quite large, although the latter do not nearly equal the size of the former. The ventral central arteries have formed more longitudinal loops similar to those shown in figure 5. They are separated more and more from each other, owing to the growth of the cord, and as this separation continues the longitudinal vessels grow in length.

In embryos of 35 to 40 mm. in length the peripheral arteries from all sides together with the lateral rami of the central arteries have formed a dense plexus in the gray substance, although the white substance contains only the peripheral arteries running through it, and the short branching rami given off at right angles from them. By the time the embryo reaches a length of 50 mm. the capillaries in the white layer have much the same appearance as those of the full term fetus, except that in the latter they branch and anastomose more freely and the growth of the cord tends to separate both the peripheral vessels and the central vessels. Embryos of 75 to 100 mm. in length show the arteries in the cord quite as completely developed as in the 240 mm. embryo.

The posterior rami of the primitive arterial tract in the ventral part of the cord of embryos of 12 to 15 mm. are more numerous than the central arteries in the 240 mm. embryo which are formed from them.

The veins within the cord develop in the same planes as the arteries, and from the same plexus of capillaries that form the latter. They may be called the central and peripheral veins corresponding to the similarly named arteries. They are shown in figures 3 and 4 in a fully developed condition.

SUMMARY

The dorsal rami of the primitive arterial tract, and other rami from the capillaries in its immediate vicinity enter the cord, forming an undifferentiated capillary plexus (fig. 5) and this plexus later becomes differentiated into arteries and veins. It was not found, as stated by Sterzi for the sheep, that each dorsal ramus of the primitive arterial tract grows into the cord, and forms a loop, giving rise to a vein which grows back along the artery to the ventral surface.

The dorsal rami of the primitive arterial tract are more numerous than the ventral central arteries which develop from them.

Sterzi reports solid blood-vessels in the cord of sheep of 5.5 mm. and hollow ones in those of 6.6 mm. In pig embryos the blood-vessels within the cord seemed to appear first as hollow vessels. These are seen first in embryos of 7.5 mm. in length.

The "tracti arteriosi laterales" of Sterzi, are the dorso-lateral arteries of this and postero-lateral of other papers, and are the posterior spinal arteries of human descriptive anatomy. Evans shows these two tracts first united by medial anastomoses in a pig of 8.5 mm. in length, but many such anastomoses are to be found in embryos as small as 7.5 mm. in the cervical and thoracic regions, and one specimen of 6.2 mm. showed them in the cervical region.

The embryos described in this paper show the mid-ventral and mid-dorsal surfaces of the cord to be covered with blood-vessels at a somewhat earlier stage than has been described.

As reported by Sterzi ('04) and Evans ('09), blood-vessels first appear on the ventro-lateral surface of the cord, then on the ventral, then on the dorso-lateral, and finally on the dorsal surface.

The blood-vessels on the cord are continuous with those in the mesenchyma surrounding it until the membranes of the cord are formed.

It is generally stated in textbooks of human anatomy that the spinal artery arises from the vertebral arteries, and is reinforced by segmental spinal arteries. It is rather to be considered that this artery arises from the segmental spinal arteries, and anastomoses with, or is reinforced by, the vertebrals.

The term median dorsal is suggested for the artery present in places in the median dorsal line of the spinal cord.

My thanks are due to Dr. Richard E. Scammon for his constant interest in the progress of this work, and for his many helpful criticisms.

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Reprinted from The Anatomical Record, Vol. 8, No. 8
August, 1914

PERSISTENT ARTERIAE BRACHII SUPERFICIALIS, ANTIBRACHII SUPERFICIALIS ET MEDIANA

E. R. HOSKINS

From the Institute of Anatomy, University of Minnesota

ONE FIGURE

An unusual artery found in the left arm of a man of thirty-seven years seems worthy of record.

The vessel emerges from the axillaris midway between the aa. subscapularis and thoracalis lateralis, on the median side. It runs in the deep fascia anterior and medial to the a. axillaris, the a. brachialis and the n. medianus, almost to the middle of the humerus, where it crosses the a. brachialis and the n. medianus, to enter the m. biceps brachii from beneath, through two large divisions.

It gives off two small cutaneous rami in the lower axillary and upper brachial regions. In size the artery is about two-thirds that of the normal subscapularis until it reaches the biceps muscle. At this point it gives rise to a small ramus almost at right angles to it. This courses lateral and anterior to the brachial artery in the deep fascia, becomes superficial at the elbow, and continues anterior to the ulna, to the palm. Here it enters into formation of the arcus volaris superficialis, together with the a. ulnaris. The arch has no connection with the a. radialis.

There is no ramus of the a. ulnaris or a. interossea which may be called an a. mediana. The a. mediana described in this paper has no relation to the n. medianus, which is placed deep in the forearm.

The embryological significance of the artery in question may be derived from Müller's[1] figure of the arteries in the arm of an

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[1] Müller '03, Anat. Hefte, Bd. 22, Taf. 25-26, fig. 9.

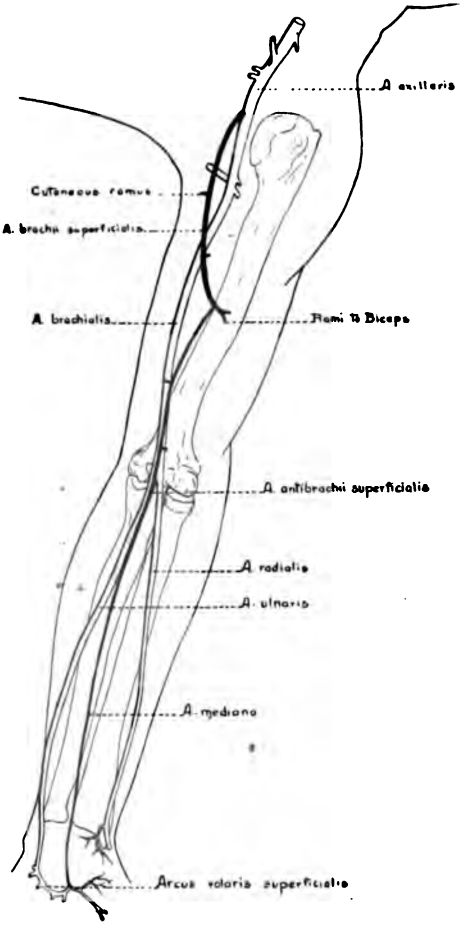

Fig. 1 Persistent arteriæ brachii superficialis, antibrachii superficialis et mediana. The rami of the aa. brachialis, radialis and ulnaris are not shown in the figure, as they are normal except for the two discrepancies noted.

11.7 mm. human embryo ('03). From this figure it would seem that we have a persistent a. brachialis superficialis, giving rise to an a. antibrachii superficialis, which becomes an a. mediana, but all anastomoses with the a. brachialis have been lost. As stated by some texts of anatomy, this condition is one that is quite rarely found.

Reprinted from THE ANATOMICAL RECORD, Vol. 9, No. 1, 1915

24. *On the development of the digitiform gland in Squalus acanthias.*

E. R. HOSKINS, Institute of Anatomy, University of Minnesota.

The digitiform gland in Aqualus is evidenced first by a slight thickening of the entoderm of the dorso-lateral border of the gut just posterior to the spiral valve. This may be seen in embryos 15 mm. in length, especially in those sectioned longitudinally. The thickening soon pushes laterally to form a hollow bud which turns and grows anteriorly along the gut. The form of the curved portion at the point of emergence from the gut always persists so that in older stages and in the adult this portion which becomes the duct of the gland enters both the intestine and the digitiform gland anteriorly.

In the stage of 28 mm. it may be seen that from the main part of the gland small buds resembling the original form of the gland grow laterally on all sides. These buds become tubules extending laterally and slightly posteriorly. They in turn give rise to secondary tubules which in time form irregular groups opening into the primary tubules. This condition is to be found throughout development, the gland becoming a compound tubular structure, the secondary tubules arising from the primary, close to the main lumen of the gland.

As the gland develops, it carries the mesentery of the intestine with it and is thus supported from the dorsal wall of the body cavity.

The entoderm of the digitiform gland is composed at first of four layers of low columnar or cuboidal cells with elongated nuclei, being similar to the entoderm of the gut from which it develops. As the gland increases in length, the epithelium is gradually reduced to one layer in thickness. Its primary and secondary tubules both arise as structures of an epithelium of one layer of cells. At the points of greatest growth, namely, at the distal ends of the tubules the nuclei are wider and shorter than along the main lumen, often being spherical.

The epithelium lining the main or central lumen later thickens giving us a structure of two layers of columnar cells with rounded nuclei in the full-term fetus and of four layers in the adult.

Morris' Anatomy, 5th edition, 1914.

INTRODUCTION

By C. M. JACKSON, M.S., M.D.

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ANATOMY, as the term is usually employed, denotes the study of the structure of the human body. Properly, however, it has a much wider significance, including within its scope not man alone, but all animal forms, and, indeed, plant forms as well; so that, when its application is limited to man, it should be qualified by the adjective human. *Human Anatomy*, then, is the study of the structure of the human body, and stands in contrast to, or rather in correlation with, *Human Physiology*, which treats of the functions of the human body, the two sciences, Anatomy and Physiology, including the complete study of man's organization and functional activities.

In the early history of the sciences these terms sufficed for all practical needs, but as knowledge grew, specialization of necessity resulted and new terms were from time to time introduced to designate special lines of anatomical inquiry. With the improvement of the microscope a new field of anatomy was opened up and the science of *Histology* came into existence, assuming control over that portion of Anatomy which dealt with the minuter details of structure. So, too, the study of the development of the various organs gradually assumed the dignity of a more or less independent study known as *Embryology*, and the study of the structural changes due to disease was included in the science of *Pathology;* so that the term Anatomy is sometimes limited to the study of the macroscopic structure of normal adult organisms.

It is clear, however, that the lines of separation between Anatomy, Histology, Embryology, and Pathology are entirely arbitrary. Microscopic anatomy necessarily grades off into macroscopic anatomy; the development of an organism is a progressive process and the later embryonic or fœtal stages shade gradually into the adult; and structural anomalies lead insensibly from the normal to the pathological domains. Furthermore it is found that in its individual development the organism passes through stages corresponding to those of its ancestry in evolution; in other words, Ontogeny repeats Phylogeny. A comprehensive study of Anatomy must therefore include more or less of the other sciences, and since an appreciation of the significance of structural details can only be obtained by combining the studies of Anatomy, including Histology and Embryology, and since, further, much light may be thrown on the significance of embryological stages by comparative studies, Anatomy, Embryology, and Comparative Anatomy form a triumvirate of sciences by which the structure of an organism, the significance of that structure, and the laws which determine it are elucidated. For this combination it is convenient to have a single term, and that which is used is *Morphology*, a word meaning literally the science of form.

In morphological comparisons, the term *homology* denotes similarity of structure, due to a common origin in the evolution of organs or parts; while *analogy* denotes merely physiological correspondence in function. Thus the arm of man and the wing of a bird are homologous, but not analogous, structures; on the other hand, the wing of a bird and the wing of an insect are analgous, but not homologous. *Serial homology* refers to corresponding parts in successive segments of the body.

Nomenclature.—Formerly there was much confusion in the anatomical nomenclature, due to the multiplicity of names and the lack of uniformity in using them. Various names were applied to the same organs and great diversity of usage prevailed, not only between various countries, but also even among authors of the same country. Recently, however, a great improvement has been made by the general adoption of an international system of anatomical nomen-

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clature. This system was first adopted by the German Anatomical Society at a meeting in Basel, in 1895, and is hence called the Basel Nomina Anatomica, or briefly, the BNA. The BNA provides each term in Latin form, which is especially desirable for international usage. Each nation, however, is expected to translate the terms into its own language, wherever it is deemed preferable for everyday usage. Thus in the present work the Anglicised form of the BNA is generally used. Where not identical, however, the Latin form is added once for each term in a place convenient for reference, and is designated by enclosure in brackets [ ]. Where necessary the older terms have also been added as synonyms.

The Commission by whom the BNA was prepared included eminent anatomists representing various European nations. The work of the Commission was very thorough and careful, and extended through a period of six years. Among the guiding principles in the difficult task of selecting the most suitable terms were the following: (1) Each part should have one name only. (2) The names should be as short and simple as possible. (3) Related structures should have similar names. (4) Adjectives should be in opposing pairs. A few exceptions were found necessary, however.

On account of its obvious merits, the BNA system has been generally adopted throughout the civilised world, and the results are very satisfactory. Comparatively few new terms have been thereby introduced, over 4000 of the 4500 names in the BNA corresponding almost exactly to older terms already in use by the English-speaking nations. Certain minor defects in the system have been criticised; but these are outweighed by the advantages of this uniform system.

Abbreviations.—Certain frequently used words in the BNA are abbreviated as follows: a., arteria (plural, aa., arteriæ); b., bursa; g., ganglion; gl., glandula; lig., ligamentum (plural, ligg., ligamenta); m., musculus (plural, mm., musculi); n., nervus (plural, nn., nervi); oss., ossis (or ossium); proc., processus; r., ramus (plural, rr., rami); v., vena (plural, vv., venæ).

Terms of position and direction.—The exact meaning of certain fundamental terms used in anatomical description must be clearly understood and kept in mind. In defining these terms, it is supposed that the human body is in an upright position, with arms at the sides and palms to the front.

The three **fundamental planes** of the body are the sagittal, the transverse and the frontal. The vertical plane through the longitudinal axis of the trunk, dividing the body into right and left halves, is the *median* or *mid-sagittal* plane; and any plane parallel to this is a *sagittal* plane. Any vertical plane at right angles to a sagittal plane, and dividing the body into front and rear portions is a *frontal* (or coronal) plane. A plane across the body at right angles to sagittal and coronal planes is a *transverse* or *horizontal* plane.

Terms pertaining to the front of the body are *anterior* or *ventral*; to the rear, *posterior* or *dorsal*; upper is designated as *superior* or *cranial*; and lower as *inferior* or *caudal*.

The term *medial* means nearer the mid-sagittal plane, and *lateral*, further from that plane. These terms should be carefully distinguished from internal (inner) and external (outer), which were formerly synonymous with them. *Internal*, as now used (BNA), means deeper, i. e., nearer the central axis of the body or part; while *external* refers to structures more superficial in position. *Proximal*, in describing a limb, refers to position nearer the trunk; while *distal* refers to a more peripheral position.

Adverbial forms are also employed, e. g., anteriorly or ventrally (forward, before); posteriorly or dorsally (backward, behind); superiorly or cranially (upward, above); and inferiorly or caudally (downward, below).

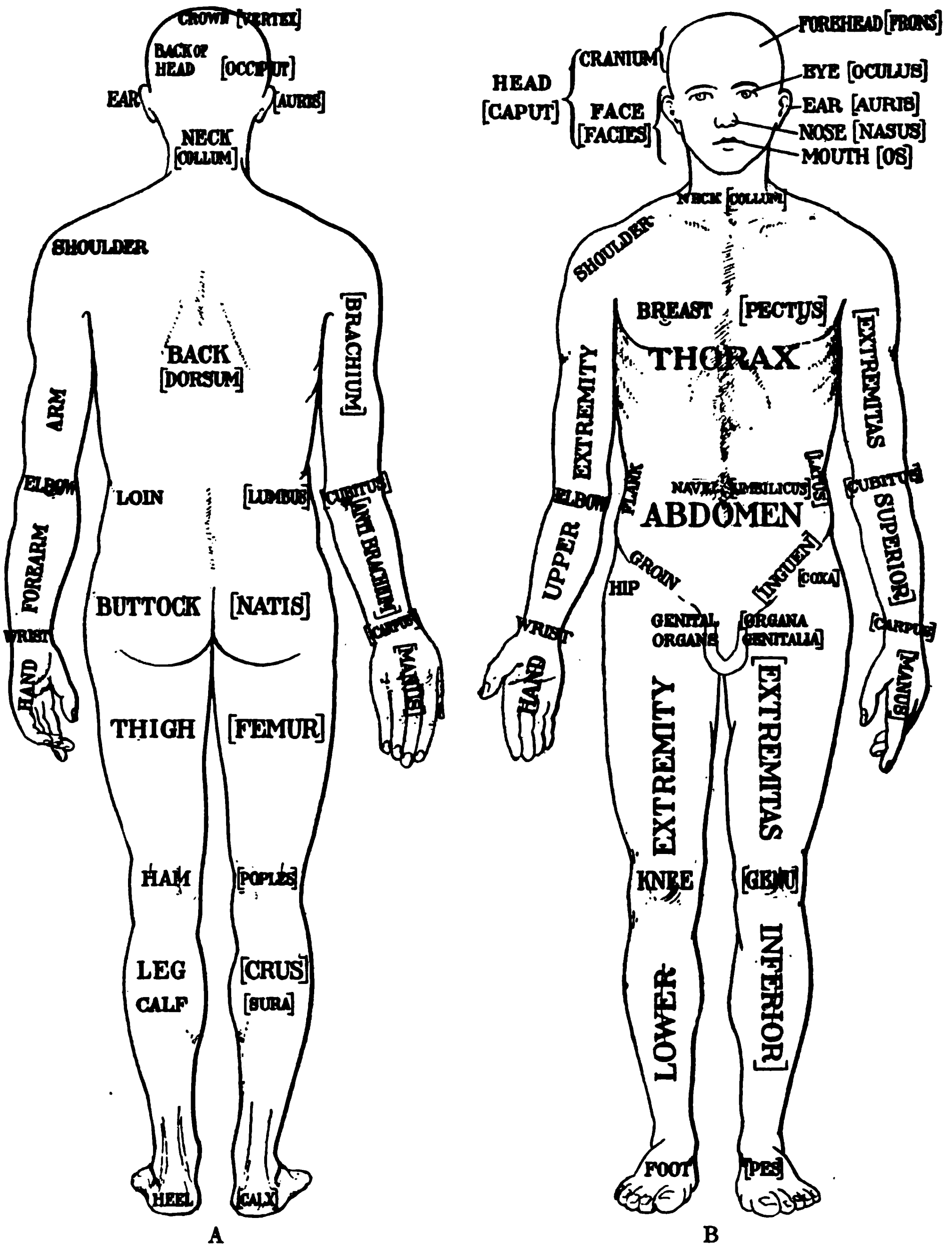
It should also be noted that the terms ventral, dorsal, cranial and caudal are independent of the body posture, and therefore apply equally well to corresponding surfaces of vertebrates in general with horizontal body axis. On this account these terms are preferable, and will doubtless ultimately supplant the terms anterior, posterior, superior and inferior.

The discrimination in the use of several similar terms of the BNA should also receive attention. Thus *medianus* (median) refers to the median plane. *Medialis* (medial) means nearer the median plane and is opposed to lateral, as above stated. *Medius* (middle) is used to designate a position between anterior and posterior, or between internal and external. Between medialis and lateralis, however, the term *intermedius* is used. Finally, *transversalis* means transverse to the body axis; *transversus*, transverse to an organ or part; and *transversarius*, pertaining to some other structure which is transverse.

Parts of the body.—The primary divisions of the human body (fig. 1) are the head, neck, trunk and extremities. The *head* [caput] includes *cranium* and *face* [facies]. The *neck* [collum] connects head and trunk. The *trunk* [truncus] includes *thorax*, *abdomen*, and *pelvis*. The *upper extremity* [extremitas superior] includes *arm* [brachium], *forearm* [antibrachium], and *hand* [manus]. The *lower extremity* [extremitas inferior] includes *thigh* [femur], *leg* [crus], and *foot* [pes].

Each of the parts mentioned has further subdivisions, as indicated in fig. 1. The cranium includes: *crown* [vertex]; *back* of the head [occiput]; *frontal* region [sinciput], including *forehead* [frons]; *temples* [tempora]; *ears* [aures], including *auricles* [auriculæ].

Fig. 1.—Parts of the Human Body. A, Posterior view. B, Anterior view.



The face includes the regions of the *eye* [oculus], *nose* [nasus], and *mouth* [os], the subdivisions of which will be given later under the appropriate sections.

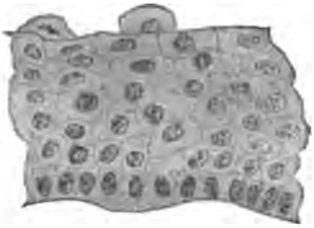
The thorax includes: *breast* [pectus]; *mammary gland* [mamma]; and *thoracic cavity* [cavum thoracis]. The *back* [dorsum] includes the vertebral column [columna vertebralis]. The abdomen includes: *navel* [umbilicus]; *flank* [latus]; *groin* [inguen]; *loin* [lumbus]; and the *abdominal cavity* [cavum abdominis]. The pelvis includes: *pelvic cavity* [cavum pelvis]; *genital organs* [organa genitalia].

buttocks [nates], separated by a cleft [crena ani] at the *anus.* The *hip* [coxa] connects the pelvis with the lower extremity.

In the lower extremity, the thigh is joined to the leg by the *knee* [genu]. The foot includes: *heel* [calx]; *sole* [planta]; *instep* [tarsus]; *metatarsus;* and five *toes* [digiti I–V], including the *great toe* [hallux] and *little toe* [digitus minimus].

The upper extremity is joined to the thorax by the *shoulder.* The arm is joined to the forearm at the *elbow* [cubitus]. The hand includes: *wrist* [carpus]; *metacarpus,* with *palm* [vola or palma] and *back* [dorsum manus]. The five *fingers* [digiti I–V] include: *thumb* [pollex], *index* finger [index]; *middle* finger [digitus medius]; *ring* finger [digitus annularis] and *little* finger [digitus minimus].

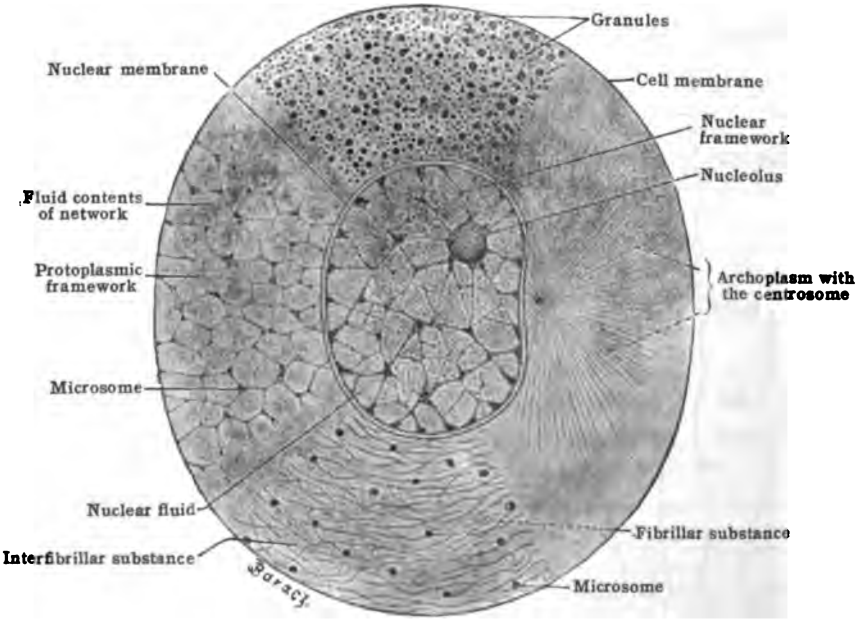
FIG. 2.—SECTION OF THE EPIDERMIS OF A FINGER, FROM A HUMAN EMBRYO OF 10.2 CM.



Organ-systems.—Each of the various parts of the body above outlined is composed of various *organs,* and the groups of related organs make up *organ-systems.*

The various organ-systems are treated as special branches of descriptive anatomy. The study of the *bones* is called *osteology;* of the *ligaments* and *joints,* *syndesmology* (or arthrology); of the *vessels, angiology*; of the *muscles, myology;* of the *nervous system, neurology*; and of the *viscera, splanchnology.* Further subdivisions are also made. The viscera, for example, include the *digestive* tract, *respiratory* tract, *urogenital* tract, etc.

FIG. 3.—DIAGRAM OF A TYPICAL CELL. (Szymonowicz.)



Tissues and cells.—The body, as above stated, has various parts, each of which may be subdivided into its component systems and organs. A further analysis reveals a continued series of structural units of gradually decreasing complexity. Thus each organ is found to consist of a number of *tissues* (epithelial, connective, muscular or nervous). Finally, each tissue is composed of a group of similar units called *cells* (figs. 2, 3) which are the ultimate structural units of the body. The body may therefore be regarded as composed of myriads of cell units, organized into units of gradually increasing complexity, very much as a social community is composed of individuals organized into trades, municipalities, etc.

Most of the individual tissues can be recognized by their gross appearance. In fact, the principal tissues were first demonstrated by Bichat through skilful dissection, maceration, etc., and without the aid of the microscope. The cellular structure of the tissues was later discovered by Schwann in 1839.

Each cell (fig. 3) is composed of a material called *protoplasm*, a viscid substance variable in appearance and exceedingly complex in chemical composition. It readily breaks down into simpler chemical compounds, whereby energy (chiefly in the form of heat and mechanical energy) is liberated. It has also the power of absorbing nutritive material to build up and replace what was lost. Its decomposition results from stimuli of various kinds, and hence it is said to be irritable. The mechanical energy which it liberates is manifested by its contractility, especially in the muscle cells. It excretes the waste products produced by its decomposition. Each cell has the power, under favourable conditions, of reproducing itself by division. Protoplasm presents, in short, all the forms of activity manifested by the body as a whole; and, indeed, the activities of the body are the sum of the activities of its constituent cells.

In the protoplasm of each cell is a specially differentiated portion, the *nucleus* (fig. 3). The nucleus plays an important part in regulating the activities of the *cytoplasm*, the general protoplasm of the cell body. The nucleus differs from the cytoplasm both structurally and chemically, and contains a very important substance, *chromatin*, which during cell division is aggregated into a definite number of masses called *chromosomes*. The cytoplasm of actively growing cells also contains the *archoplasm* and *centrosome*, structures of importance in the process of cell division. Further details concerning the cells and tissues may be found in the text-books of cytology and histology.

In earlier days Human Anatomy was almost entirely a descriptive science, but little attention being paid to the significance of structure, except in so far as it could be correlated with physiological phenomena as they were at the time understood. In recent years attention has been largely paid to the morphology of the human body and much valuable information as to the meaning of the structure and relations of the various organs has resulted. Since the form and structure of the body are the final result of a series of complicated developmental changes, the science of Embryology has greatly contributed to our present knowledge of human Morphology; and, accordingly, a brief sketch of some of the more important phases of morphogenesis will form a fitting introduction to the study of the adult.

References.—*General:* For looking up the literature upon any anatomical topic, the best guide is the "Jahresbericht ueber die Fortschritte der Anatomie und Entwicklungsgeschichte," which contains classified titles and brief abstracts of the more important papers in gross anatomy, histology and embryology. Other useful aids are the "Zentralblatt fuer normale Anatomie," the "Index Medicus" and the catalogue of the Surgeon Genera 's Library of the War Dep't. (Washington, D. C.). The latter two contain titles only, but cover the whole field of medicine. The "Concilium Bibliographicum" also provides a convenient card-index system of references for the biological sciences, including Anatomy.

For *nomenclature:* His, Archiv f. Anat., 1895 (BNA system); Barker, Anatomical Nomenclature. *Cells and tissues:* Wilson, The Cell; Hertwig, Zelle und Gewebe (also English transl.); Schaefer, Microscopic Anatomy (in Quain's Anatomy, 11th ed.); Heidenhain, Plasma und Zelle.

SECTION I

MORPHOGENESIS

REVISED FOR THE FIFTH EDITION

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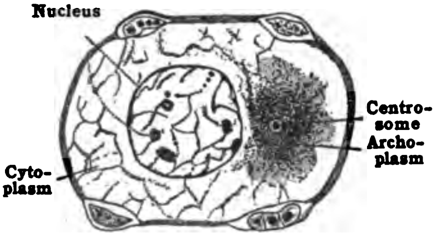
CHANGE is a fundamental characteristic of all living things. The human body during its life cycle accordingly passes through various phases of form and structure. In the earliest embryonic phases of development the changes are very rapid, decreasing in rapidity during the later fœtal stages, but continuing at a diminishing rate throughout infancy, childhood and youth up to the adult. Following the acme of maturity, changes continue which lead gradually to senescence and final death of the body.

This cycle of change in the body depends upon similar changes in its various component organs, each having its own characteristic life cycle. In a few of the organs this cycle is very short, as in some of the organs of the embryo (e. g., mesonephros). Other organs persist only during childhood (e. g., thymus); while the majority continue, with varying degrees of change, throughout postnatal life. The final death of the body is due to the breakdown of some of the essential organs.

A further analysis reveals the fact that the characteristic life cycles of the organs depend ultimately upon similar changes in their constituent tissues and cells. Every cell has a definite life cycle, an early period characterised by rapid and vigourous changes, later periods of differentiation and maturity, followed by stages of degeneration and death. This cycle of cell changes has been designated by Minot as *cytomorphosis*.

Growth.—Associated with the process of cell differentiation (cytomorphosis), and even more important as a factor in the morphogenesis of the body, is the process of *growth*. The developmental changes in form and structure of the body are due largely to the unequal growth of its various parts. Growth, like other changes in the body and its parts, depends ultimately upon the characteristics of the constituent cells.

FIG. 4.—THE OVUM OF A NEW-BORN CHILD, WITH FOLLICLE CELLS. (After Mertens.)



The cell changes during growth may be grouped under two heads. The first, or growth proper, involves merely the *enlargement* (hypertrophy) of the individual cells and intercellular products. The second includes the *multiplication* (hyperplasia) of the cells, which is accomplished by mitotic division. Cell division is necessary in cell growth, for otherwise the cell would soon reach a size where its surface (for nutritive, respiratory and excretory purposes) would be inadequate for its mass. In general, however, cell division is most active in the earlier embryonic periods, during which the cells remain small. Later, cell division diminishes or ceases, and growth is due chiefly to enlargement of the cells already present. It is also during the later period, when the cells have ceased rapid division, that the process of cell differentiation and tissue formation is most marked.

The principle of the *ratio of surface to mass* often applies to the growing organs as well as to the individual cells. To maintain the necessary ratio, the surface area is increased by the formation, through localised unequal growth, of *projections* (e. g., villi or folds) or *invaginations* (e. g., glands) from surfaces. Innumerable modifications of this principle occur throughout the process of morphogenesis.

FIG. 5.—OVUM FROM OVARY OF A WOMAN THIRTY YEARS OF AGE. *cr*, corona radiata. *n*, nucleus. *y*, yolk. *p*, clear protoplasmic zone, *ps*, perivitelline space. *zp*, zona pellucida. (McMurrich's Embryology, from Nagel.)

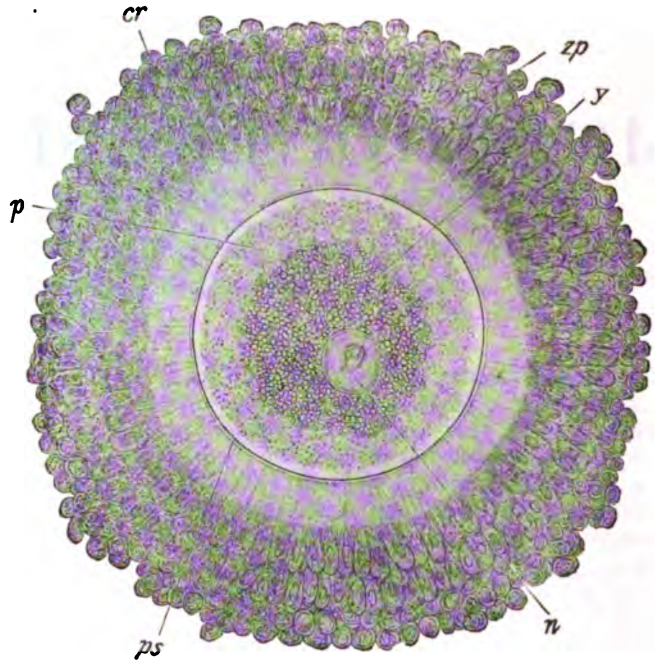
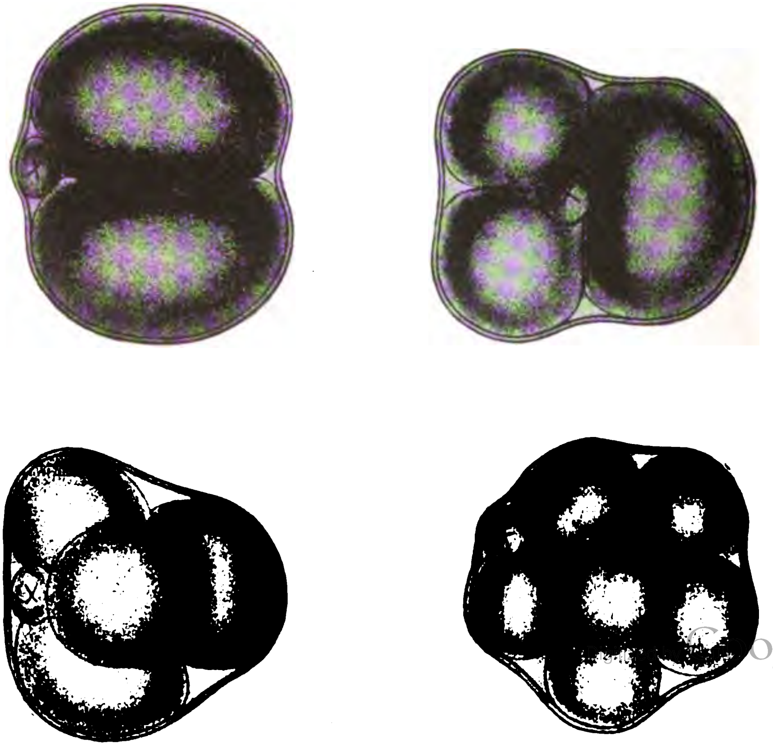


FIG. 6.—STAGES OF SEGMENTATION IN THE OVUM OF THE MOUSE. *x*, polar body. (McMurrich's Embryology, from Sobotta.)



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While the present work deals primarily with the adult human organism in the stage of maturity, reference is made also to its changes according to age. Although these changes for the various systems of organs are described under the appropriate sections, it is desirable to consider first some of the more fundamental features pertaining to the body as a whole. This applies particularly to the earlier embryonic period, which includes the more general phases of morphogenesis. No attempt will be made to describe fully the process of development, the details of which are to be found in text-books of embryology.

Segmentation of the ovum.—The human body, like all living organisms, arises from a single cell, the egg-cell or *ovum*. An early stage in the development of the ovum is shown in fig. 4, and a later stage, approaching maturity, in fig. 5. The mature human ovum is about 0.2 mm. in diameter. In the uterine (Fallopian) tube, the fertilised ovum undergoes segmentation, the various stages of which are represented in figs. 6 and 7.

FIG. 7.—DIAGRAM OF SECTION THROUGH A MAMMALIAN OVUM AT THE MORULA STAGE.

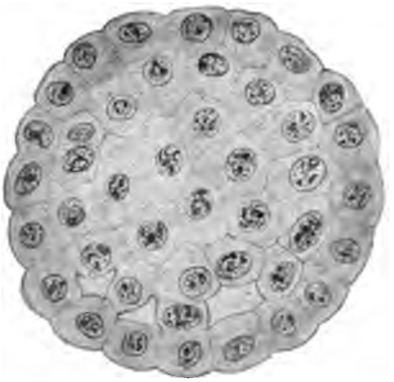
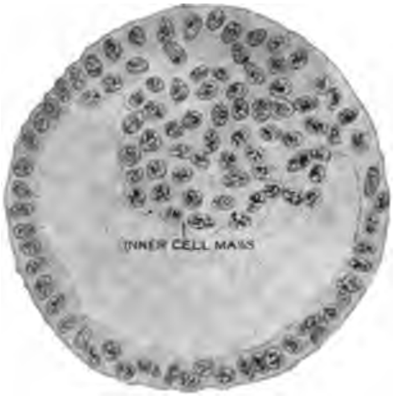


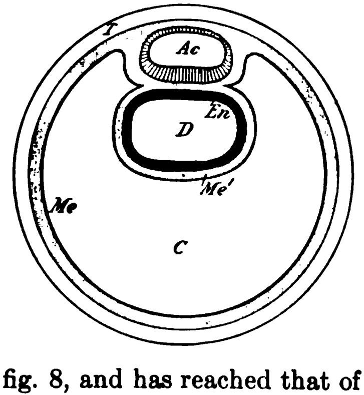
FIG. 8.—DIAGRAM OF SECTION OF A MAMMALIAN OVUM SHOWING THE INNER CELL MASS.



While the processes of maturation, fertilisation and segmentation have not as yet been observed in the human ovum, the evidence of comparative anatomy makes it very probable that in all essential respects these processes are like those found in other mammals. As a result of the successive divisions of the ovum in segmentation, a spherical mass of cells, the *morula* (fig. 7) is formed. In this mass, an excentric cavity forms (fig. 8) whereby the mass is transformed into a hollow vesicle. The wall of this vesicle is probably formed throughout the greater part of its extent by a single layer of cells; but at one point of the circumference there is a group of cells termed the *inner cell mass* (fig. 8). Probably about this time the ovum enters the uterine cavity, and through the activity of the outer layer of cells (*trophoblast*) becomes embedded in the uterine mucosa.

Formation of the embryonic disc and germ layers.—In the earliest human embryos which have been described, development has already proceeded beyond the stage represented by fig. 8, and has reached that of fig. 9. Within the inner

FIG. 9.—DIAGRAM SHOWING THE RELATIONS OF THE GERM LAYERS IN AN EARLY EMBRYO. *Ac*, amniotic cavity, lined by ectoderm. *D*, yolk-sac, lined by endoderm (*En*). *Me,Me'*, mesoderm, *C*, extra-embryonic cœlom. *B*, chorion. *T*, trophoblast. (McMurrich.)

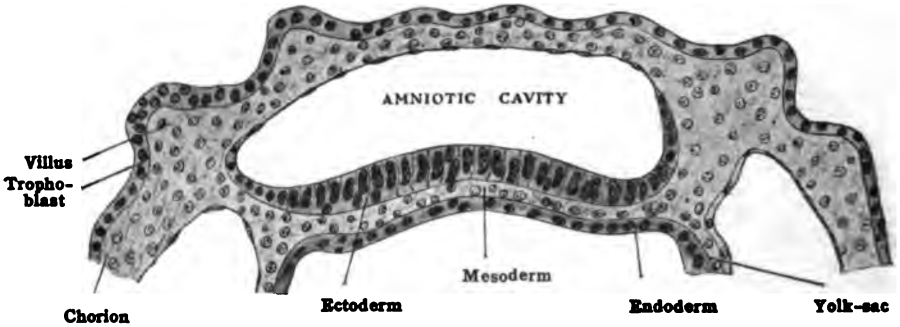


cavity; the deeper (D) is the cavity of the *yolk-sac;* while between them is a plate of cells forming the *embryonic disc.* The embryonic disc (figs. 9 and 10) contains three layers of cells,—the fundamental *germ layers,—ectoderm* (Ec), *endoderm* (En), and *mesoderm.*

The germ layers of the embryonic disc are of prime importance in the development of the body. From the *ectoderm,* which lies next to the amniotic cavity and represents the upper (later outer) germ layer, are derived the epidermis and the entire nervous system. From the *endoderm,* which lies next to the yolk-sac, and represents the lower (later inner) germ layer, is derived the epithelial lining of the digestive mucosa and its derivatives. From the *mesoderm,* or middle germ layer, is differentiated the remainder of the body, including the skeletal and supporting tissues, vascular system, muscle and most of the urogenital organs.

The germ layers also extend beyond the embryonic disc, as shown in figs. 9 and 10. The *yolk-sac* is made up of a lining of endoderm and an outer layer of mesoderm. The *amnion,* which later becomes separated from the chorion, is composed of mesoderm lined by endoderm. The outer cell layers form the *chorion,* which likewise shows two layers, the outermost of which (trophoblast) is ectoderm, the inner, mesoderm. In fig. 10 the chorion is beginning to send out root-like projections (villi) which invade the uterine mucosa.

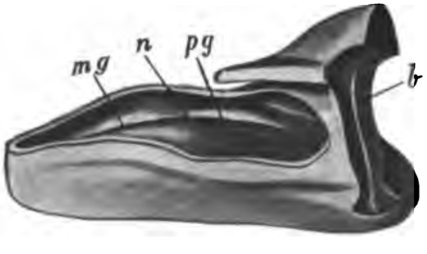
Fig. 10.—Diagram of Section of a Mammalian Ovum showing the Embryonic Disc, Amniotic Cavity and the Germ Layers.



It is thus noteworthy that of the cells derived from the ovum relatively only a few—those of the embryonic disc—enter directly into the formation of the body. The yolk-sac, a rudimentary organ of phylogenetic significance, is later chiefly absorbed, although the proximal portion may enter slightly into the formation of the intestinal wall. The amnion is a protective membrane, while the chorion forms the fœtal part of the placenta.

Development of the embryonic disc.—When first formed, the surface of the embryonic disc shows no trace of differentiation. A slightly later but still comparatively early stage in its development is shown in fig. 11. It is here viewed from above, the amnion having been removed. The disc is an elliptical plate, whose long axis represents the mid-line of the embryo. Near the center is a small rounded depression, the *primitive pit.* Extending backward (toward the tail end of the embryo) from this is a dark line, the *primitive streak,* corresponding to a groove, the *primitive groove.* Extending forward from the primitive pit is an indistinct wide shallow groove, the *neural groove.*

Fig. 11.—Model Showing the Embryonic Disc from an Embryo 1.17 mm. In Length. Viewed from above and laterally, the roof of the amniotic cavity having been removed. *n,* primitive pit (neurenteric canal). *pg,* primitive groove. *mg,* neural groove. *b,* body-stalk. (McMurrich, from Frassi.)

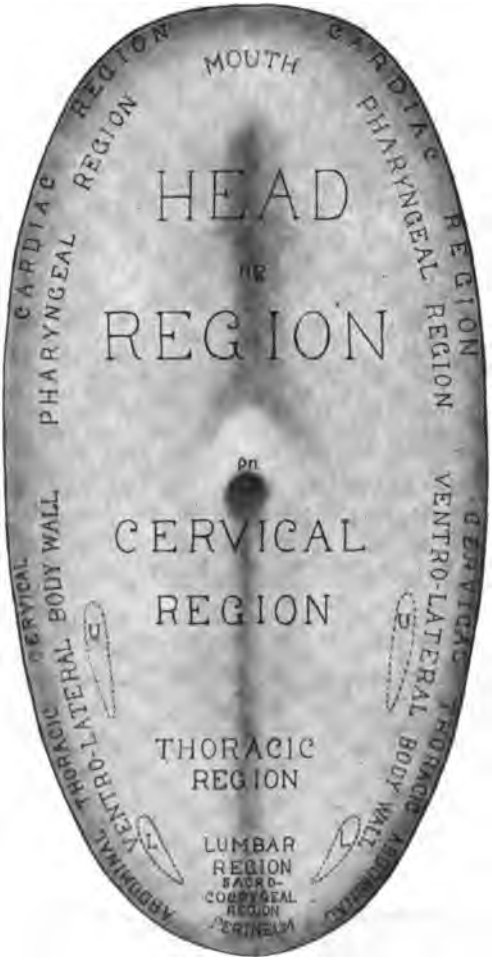


At an earlier stage, the primitive streak extends further forward, possibly to the anterior end of the embryonic disc (Spee). The primitive streak and groove probably correspond to the fused lips of a primitive blastopore. They represent a centre of proliferation from which the mesoderm is budded off from the ectoderm and spreads out to form the middle germ layer of the embryonic disc.

At the anterior end of the primitive streak this proliferation extends forward as a plate of cells, the so-called '*head process*.' The axial portion of this process is the anlage of the *notochord*, the embryonic skeletal axis. It contains a canal, which opens into the primitive pit. The notochordal anlage soon fuses with the underlying endoderm, and its canal forms the transient *neurenteric canal*.

In the mid-line anterior to the primitive streak there appears the shallow *neural groove* (fig. 11), corresponding to a thickened plate of ectodermic cells, the *neural plate*. The neural groove is slightly forked at its posterior extremity, in the region of the *primitive node* (Hensen's node), which forms the dorsal lip of the primitive pit. As development proceeds, the neural plate extends posteriorly, and the primitive pit is accordingly shifted backward, the corresponding part of the primitive groove being converted into 'head process.' The primitive streak thus becomes progressively shortened (cf. figs. 11 and 13).

FIG. 12.—TOPOGRAPHY OF THE EMBRYONIC DISC. DIAGRAM OF RELATIONS AT THE LENGTH OF ABOUT 1 MM. *ng*, neural groove. *pn*, primitive node. *pp*, primitive pit. *U*, upper limb. *L*, lower limb.



Topography of the embryonic disc.—Although only slight signs of differentiation are visible in the embryonic disc at the stage shown in fig. 11, it is already possible to map out more or less definite areas corresponding to all the various regions of the future body, as shown in fig. 12.

Beginning anteriorly, the head region is relatively enormous in size, occupying at this time the entire portion in front of the primitive pit and forming about half of the entire disc. The cervical, thoracic, lumbar and sacro-coccygeal regions appear successively smaller, approaching the posterior end ('tail bud') of the primitive streak. It is also a striking fact that the future dorsal region of the body wall, corresponding to the central portion of the disc, along each side of the mid-line, is now larger than the ventro-lateral regions, which occupy a

The topography of the germinal areas in the embryonic disc shown in fig. 12 is based partly upon a study of the succeeding stages of development, and partly upon the results of experiments upon the germinal disc in lower forms, especially in the chick (Assheton, Peebles, Kopsch).

Law of developmental direction.—In the relative size of the various embryonic areas is foreshadowed what may be termed the law of direction in development. In general it is found that development (including growth and differentiation) in the long axis of the body appears first in the head region and progresses toward the tail region. Similarly in the transverse plane development begins in the mid-dorsal region and progresses latero-ventrally (in the limbs, proximo-distally). These principles are of great importance in morphogenesis.

Fig. 13.—Human Embryo 1.54 mm. long. Viewed from above, the roof of the amniotic cavity having been removed. (Minot, after Graf Spee.)

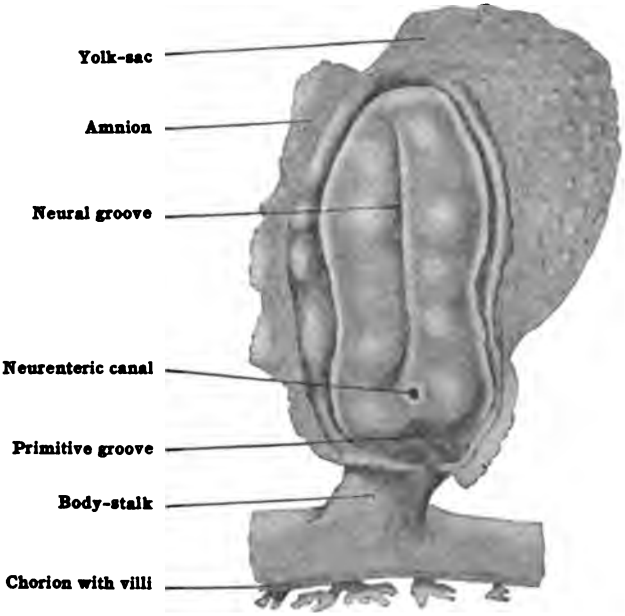
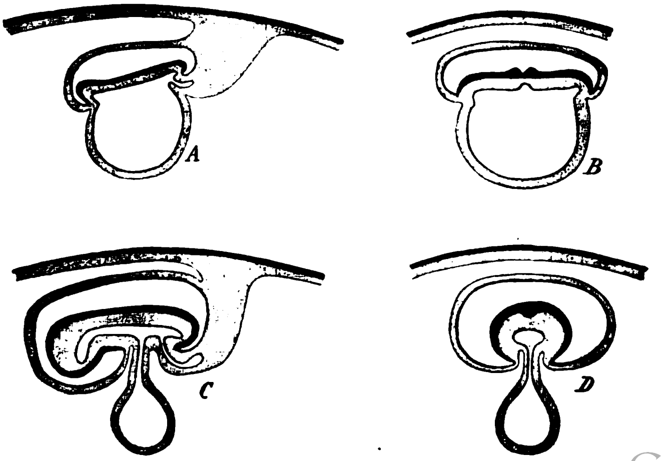


Fig. 14.—Diagrams Showing the Constriction of the Embryo from the Yolk-sac. *A* and *C*, longitudinal sections; *B* and *D*, corresponding cross-sections. (McMurrich.)

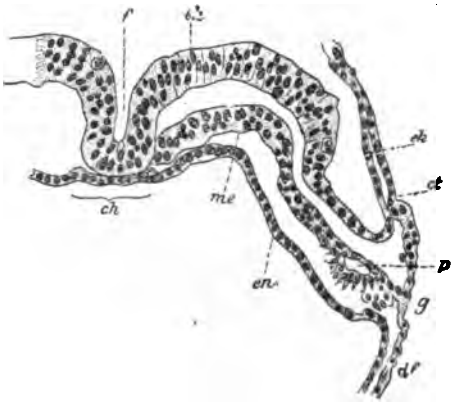


The law of developmental direction is also probably of phylogenetic significance. The *cranio-caudal* direction of development is in accordance with the theory that the head is the most primitive portion of the body, and hence precocious in development. The trunk is perhaps a secondary acquisition, hence arising as an extension of the primitive head region.

The *dorso-ventral* direction of development, together with the plate-like form of the embryonic disc, has a different phylogenetic significance. Both are probably inherited from an ancestral type with a yolk-laden ovum. In such an ovum, with the meroblastic type of segmentation, the flattened embryonic disc gradually spreads from the dorsal surface in a ventral direction around the underlying yolk-mass.

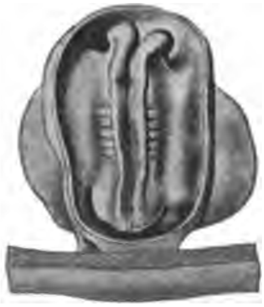
Derivation of body tube from embryonic disc.—The primary result of the precocious growth in the dorsal region of the embryonic disc is the conversion of the disc into the body tube, curved ventrally in its long axis (fig. 14).

FIG. 15.—PORTION OF CROSS SECTION OF THE EMBRYO SHOWN IN FIG. 13. *ch*, notochord. *ct*, somatic mesoderm. *df*, splanchnic mesoderm. *g*, junction of extra-embryonic somatic and splanchnic mesoderm. *ek*, ectoderm. *en*, endoderm. *me*, embryonic mesoderm. *f*, neural groove, *p*, beginning of embryonic cœlom (pericardial cavity). (Minot, after Graf Spee.)



As a result of the more rapid expansion of the germ layers (especially the ectoderm) near the mid-line, the dorsal surface of the embryonic disc in general becomes convex, with a depression laterally (where growth is less rapid) forming a groove at the line of attachment of the amnion (figs. 11, 12, 13, 14 B). The unequal growth in the germ layers is clearly evident in the cross section shown in fig. 15. By a continuation of this process, the margins of the embryonic disc become still further depressed and finally folded in ventrally so as to transform the disc into a tube (fig. 14 D). Similarly, by a more rapid expansion of the dorsal layer of the disc in the longitudinal axis, the head and tail ends of the disc are folded and tucked in ventrally, and the primitive body tube is thus correspondingly curved in its long axis (figs. 14 A, 14 C).

FIG. 16.—MODEL OF HUMAN EMBRYO 1.8 MM. LONG. Viewed from above, the roof of the amniotic cavity having veen removed. Near the caudal end of the neural groove, the primitive pit (opening of neurenteric canal) is visible. The primitive somites are appearing in the occipital region, the fourth corresponding to the boundary between head and neck. (McMurrich, from Keibel and Elze.)



The embryonic disc is thus converted into a tube composed of an outer layer of ectoderm, a middle layer of mesoderm and an inner layer of endoderm. The yolk-sac now presents an expanded *yolk-vesicle* lined by endoderm which is still continuous through the constricted *yolk-stalk* with the endoderm lining the primitive *enteric cavity* (fig. 14 C). The enteric cavity (or archenteron) has a blind tubular prolongation (fore gut) into the head region, and another (hind gut) into the tail region. From the latter a slender diverticulum, the *allantois*, extends into the body stalk (later the umbilical cord). The allantois is an organ of phylogenetic importance, with which the urinary bladder is later connected.

Formation of the neural tube.—The principle of unequal growth applies to the formation not only of the body as a whole, but also of its constituent parts. Thus the anlage of the nervous system arises from the ectoderm as a wide groove whose edges (neural ridges) by local growth are folded upward so as to meet in the mid-line where they fuse, thus transforming the groove into the *neural tube* (figs. 11, 12, 13, 15, 16, 17, 18).

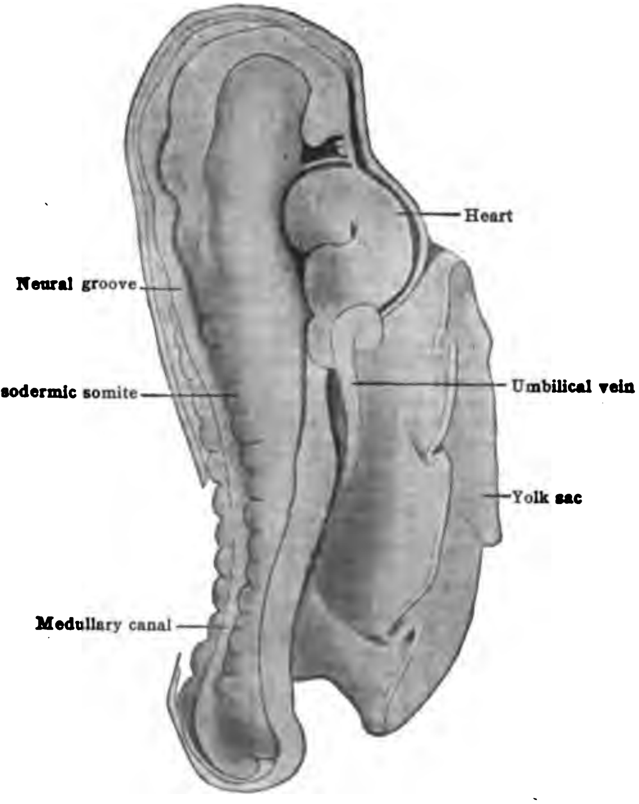
The closure begins, not at the anterior end (as might be expected from the general law of cranio-caudal development), but in the cervical region, extending forward into the brain region, and backward along the spinal cord. Thus the extreme ends (anterior and posterior neuropores) are the last to close.

The precocious and energetic growth of the neural anlage is largely responsible for the ventral flexure of the embryonic body axis, especially in the head region, where the flexures of the brain are very conspicuous (figs. 22, 26).

With the closure of the neural tube dorsally and of the alimentary canal ventrally the human embryo assumes the typical vertebrate form. The cylindrical body wall now encloses two tubes (neural and enteric) with the longitudinal axis (notochord) between them (figs. 18, 24).

After the embryonic disc has been transformed into a tube, the body of the human embryo in cross section appears not circular but elongated dorso-ventrally. This is the typical form for vertebrates with horizontal body axis. In later fœtal stages, the body becomes more rounded in cross section, and finally, with the assumption of the erect posture in postnatal life, becomes decidedly flattened dorso-ventrally (figs. 20, 21).

Fig. 17.—A Human Embryo 2.5 mm. in Length. (After Kollmann.)



Development of the mesoderm.—The mesodermic layer on each side of the notochord in the embryonic disc develops in two divisions. The medial (or dorsal) divisions form a series of hollow segments, the *somites* (figs. 16, 17, 18). The lateral (later ventral) divisions each split into an upper (outer) or somatic layer and a lower (inner) or visceral layer. When the embryonic disc becomes folded, the corresponding somatic and visceral layers unite ventrally and enclose between them the common *cœlom* or primitive body cavity (fig. 18).

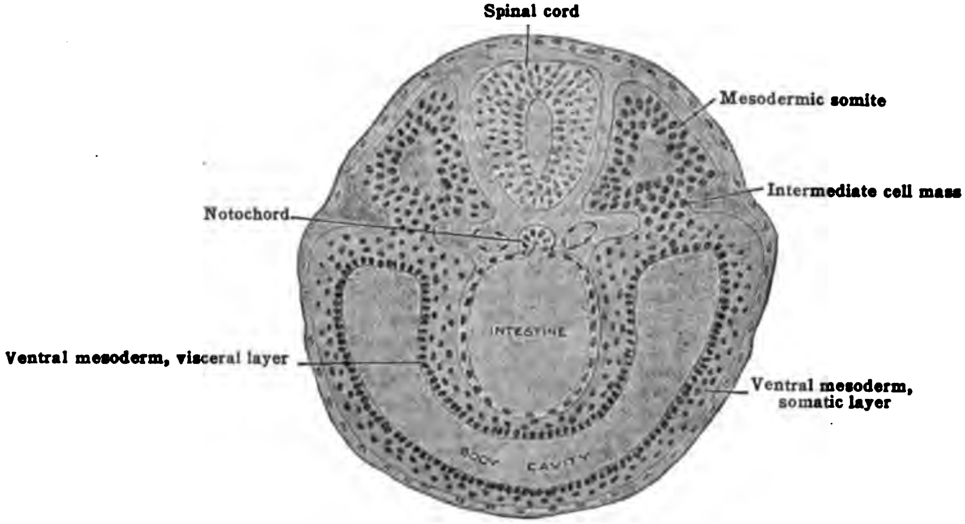
As previously noted, the mesoderm arises chiefly from the lateral portions of the 'head process.' A comparatively early stage before the appearance of the somites is shown in cross section in fig. 15. The somites appear first in the occipital region, and rapidly differentiate successively in the cranio-caudal direction (figs. 16, 17, 22). In embryos 7 or 8 mm. in length, about 40 somites may be distinguished, 3 to 5 occipital, 8 cervical, 12 thoracic, 5 lumbar, 5 sacral and 5 or 6 coccygeal (in the rudimentary tail region).

The *cœlom* or body cavity is unsegmented. Two primitive pericardial cavities appear separate at first, but soon fuse and unite with the general cœlom. Later the general cœlom becomes secondarily divided into the permanent pericardial, pleural and peritoneal cavities.

The outer layer of the lateral mesodermic division forms the somatic or parietal layer of the peritoneum, etc. The inner layer forms the visceral or splanchnic layer, and develops not only the serous membrane, but also the muscular and connective tissue of the walls of the alimentary canal and its derivatives.

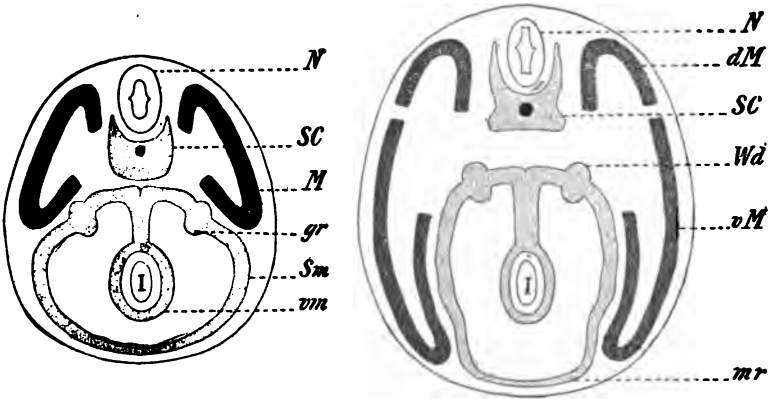
Development of the somites. Metamerism.—The appearance of the somites marks the beginning of *metamerism*, the arrangement of the body in successive segments or metameres. Each somite develops a primitive muscle segment, *myotome*, and a skeletal segment, *sclerotome* (figs. 18, 19). Moreover, the corresponding nerves and blood-vessels likewise assume a metameric arrangement. This metamerism persists (more or less modified) in the adult neck and trunk.

Fig. 18.—Diagram of a Cross Section of a Human Embryo.



The differentiation of the somites is illustrated by figs. 18 and 19. The medial wall of each somite forms the *sclerotome*. Its cells migrate to form the corresponding vertebra, rib, etc., as well as the *mesenchyme* forming the various connective tissues in this region. The remainder of the somite forms the *myotome*, from which the voluntary musculature of the trunk, the neck and (in part) the head is derived. The dorsal portions of the myotomes develop the muscle in the dorsal region of the trunk, while the ventral portions extend ventralward to form the musculature of the latero-ventral body walls (figs. 19, 20, 21, 23).

Fig. 19.—Diagrams Illustrating the History of the Mesoderm. *M*, myotome, *dM*, dorsal portion of myotome. *vM*, ventral portion of myotome. *SC*, sclerotome. *gr*, genital ridge. *Wd*, Wolffian duct. *Sm*, somatic layer of mesoderm. *vm*, visceral layer of mesoderm. *mr*, membrana reuniens. *I*, intestine. *N*, neural tube. (McMurrich.)

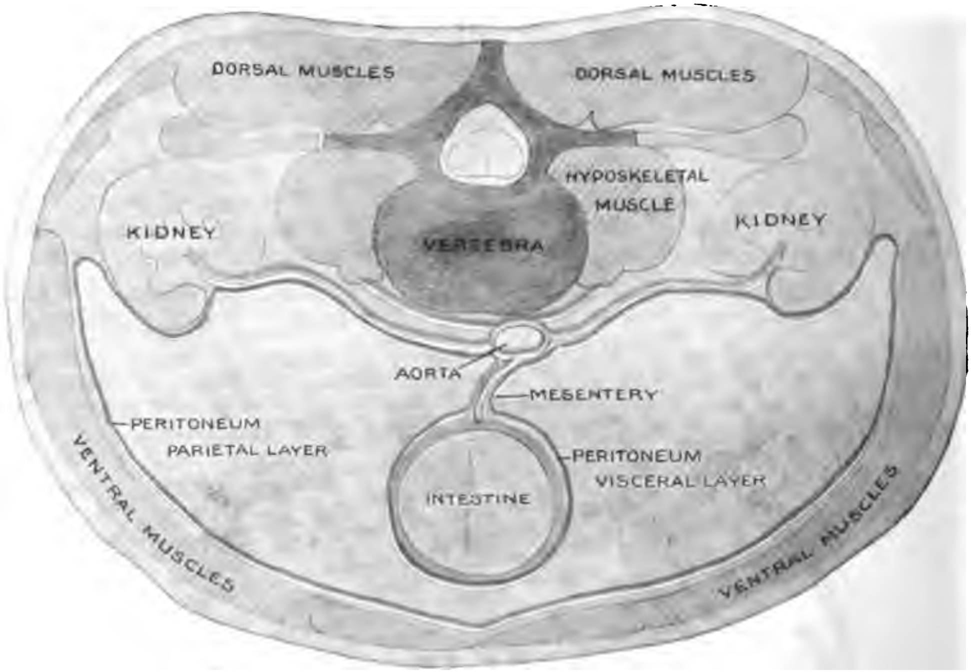


At the junction of the dorsal and ventral divisions of the mesoblast is a group of cells called the *intermediate cell mass*. This mass becomes segmented (corresponding to the somites) and each segment, or *nephrotome*, gives rise to a portion of the *mesonephros*, the provisional kidney. Other cells of the mass become *mesenchyme*, which is converted into blood-vessels, connective tissue, etc.

As development proceeds, the metamerism of the muscles and arteries becomes more or less obscured, but that of the vertebræ and nerves is fully retained even in the adult. In the case of the muscle plates, from which all the voluntary musculature of the trunk is derived, great modifications occur. Extensive fusion of successive plates occurs, the intervening connective tissue disappearing more or less completely; associated with this fusion there is longitudinal and tangential splitting of the somites to form individual muscles; and portions of some of the plates may wander far from their original position. But notwithstanding these complicated changes, indications of the primary metameric arrangement of the muscle plates are abundant, and even in the most extreme cases of modification the developmental history of a muscle can be determined by means of its nerve supply. For the fibres derived from each plate will usually retain, no matter what changes of independence or position they may undergo, the innervation by their originally corresponding segmental nerve; so that the occurence in the lumbar region of the body of muscle-fibres (the diaphragm) supplied by nerve-fibres from a cervical nerve is evidence that the muscle-fibres have been derived from a cervical mesodermic somite and have subsequently migrated to the position they finally occupy.

As regards the arteries, they arise primarily from a longitudinal stem, the aorta, in a strictly segmental manner, each metamere having distributed to it two pairs of arteries and a single median one (fig. 20). One pair of arteries supplies the body wall, and these retain very distinctly their original metameric arrangement; the other pair passes to the paired viscera, such as the lungs, kidneys, ovaries (or testes), so many of the pairs disappearing, however, that their metameric arrangement is not very evident in the adult. The unpaired vessels supply the digestive tract and its unpaired appendages, such as the liver and pancreas, and undergo great modifications, those of the lower thoracic and lumbar regions becoming reduced by fusion and degeneration to three main trunks.

FIG. 20.—DIAGRAM OF A TRANSVERSE SECTION THROUGH THE ABDOMINAL REGION.



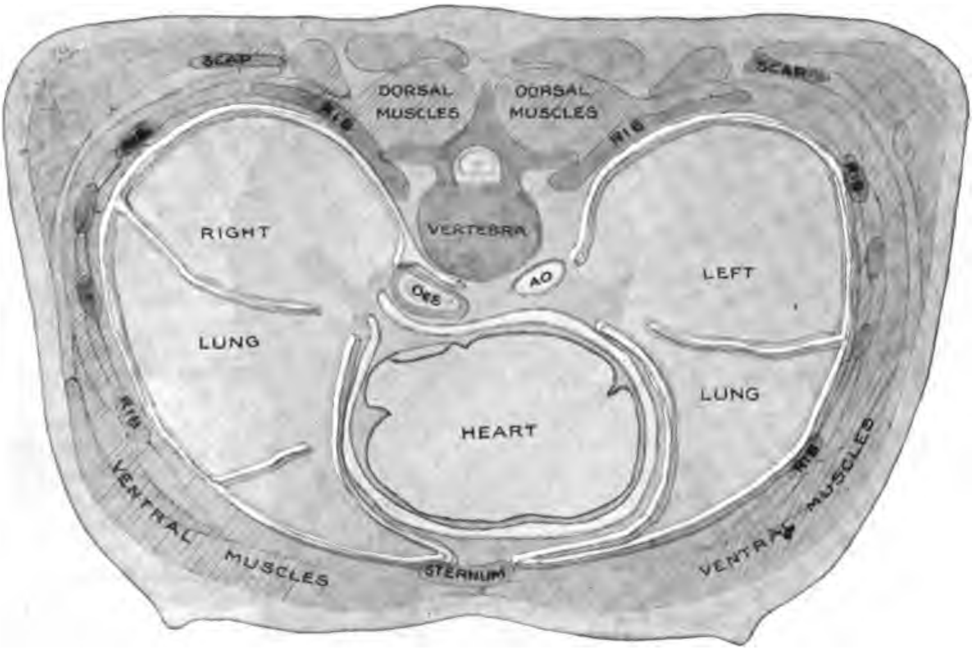
Branchiomerism.—Throughout the trunk and neck regions, then, a fundamental metameric plan underlies and determines the arrangement of many parts. In the head there is also evident a primary arrangement of the parts in succession; but this arrangement appears to be somewhat different from that of the trunk in that it involves the ventral instead of the dorsal mesoderm and is associated with the occurrence of branchial arches rather than with true mesodermic somites. It is consequently termed *branchiomerism.*

Not but that there are also indications of metamerism in the head, the muscles of the orbit, and the majority of the extrinsic muscles of the tongue, together with the nerves supplying these muscles, being apparently metameric structures, but the metamerism of this region of the body is largely overshadowed by the branchiomerism.

If an embryo of about the fifth week of development (fig. 22) be examined, there will be observed on the surface of the body in the pharyngeal region three or four linear depressions, and sections will show that similar and corresponding grooves also occur upon the inner surface of the pharyngeal wall. These are the *branchial grooves*, and since they are four in number (with a rudimentary fifth) in the human embryo, they mark off five *branchial* (*or visceral*) *arches*, the first of which lies between the oral depression and the first branchial groove, while the fifth is situated behind the fourth groove. These branchial arches are so named because they represent the arches which (excepting the first) support the gills (branchiæ) in the lower vertebrates, the grooves representing the branchial slits, even although they do not become perforated in the human embryo.

Each *branchiomere* consists of an axial skeletal structure, of muscles which act on this skeleton, of a nerve which supplies the muscles and the neighbouring integument and mucous membrane, and of an artery which carries blood to all these structures. The arches, however, do not in the human embryo retain their original branchial function, but undergo extensive modifications, becoming adapted to various functions and showing less in the adult of their originally simple arrangement than do the metameres. Nevertheless no matter what modifications the musculature of any arch may undergo, it will retain its original innervation and, to a large extent, its relations to the skeletal elements of its arch; and even the arteries in their distribution show clear indications of being arranged in correspondence to the various arches.

FIG. 21.—DIAGRAM OF A TRANSVERSE SECTION THROUGH THE THORACIC REGION. (The pleura is represented in blue and the pericardium in red.)



With respect to the fate of the various structures pertaining to each branchial arch, their general arrangement in the adult body may be stated in the following table:—

RELATIONS OF THE BRANCHIAL ARCHES IN THE ADULT

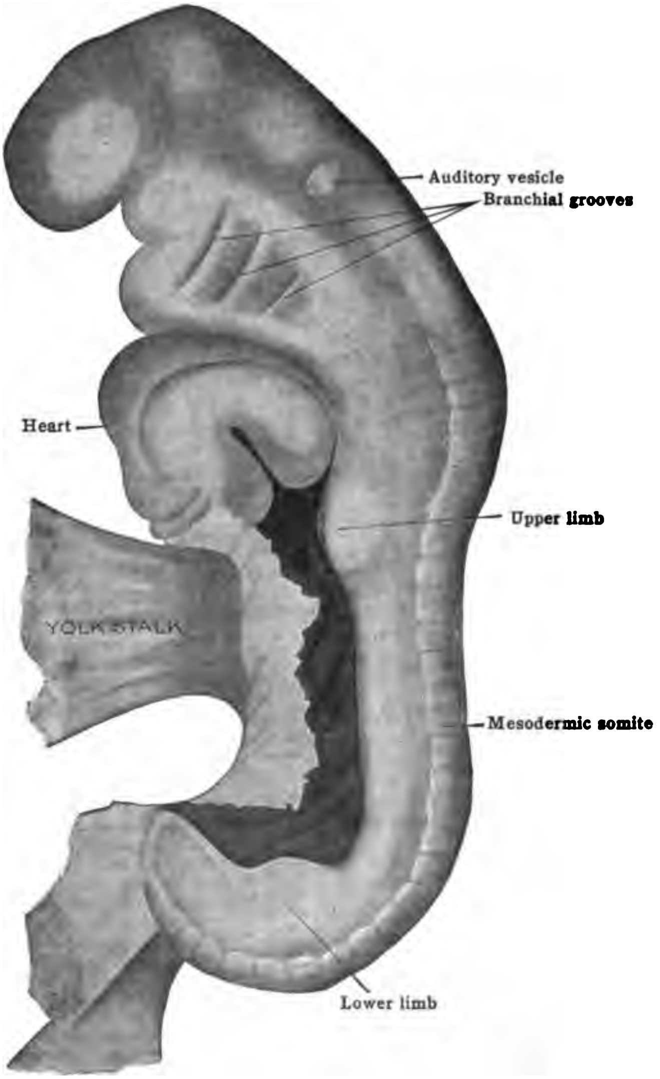
Arch	Skeleton	Muscles	Nerve
First arch.......	Mandible, malleus and incus.	Masticatory, mylohoid and digastric (ant.), tensor tympani.	Trigeminus.
Second arch.....	Hyoid (lesser cornu), styloid process and stapes.	Stylohyoid, digastric (post.), muscles of expression, stapedius.	Facialis.
Third arch......	Hyoid (greater cornu)...	Pharyngeal.................	Glossopharyngeus.
Fourth and fifth arches.	Thyreoid cartilage......	Pharyngeal and laryngeal.....	Vagus.

Branchial grooves.—Of the *external branchial grooves*, the first (lying between mandibular and hyoid arches) becomes deepened to form the *external auditory meatus*, the margins becoming elevated to form the *auricle* (fig. 26). The region corresponding to the second, third and fourth external grooves becomes depressed, forming the *sinus cervicalis*, which soon closes up and disappears.

The *internal branchial grooves* or pouches communicate with the pharyngeal cavity and are lined with endoderm. The first internal groove becomes transformed into the *auditory* (Eustachian) *tube, tympanic cavity,* etc. The second internal groove persists in part as the fossa of the *palatine tonsil.* The third and fourth grooves are probably represented in part by the *vallecula* and *recessus piriformis,* detached portions of their lining endoderm giving rise to the *thymus, parathyreoid glands,* etc. The rudimentary fifth groove is said to give rise to the *ultimobranchial body,* a structure of uncertain significance (fig. 27).

Development of the face.—The facial region is at first relatively small. It includes the sense organs (eye, ear, nose) and mouth region. Some of the more important developmental features may be briefly mentioned. In an embryo of the sixth week (fig. 28) the wide mouth aperture is seen to be bounded below (posteriorly) by the lower (*mandibular*) portion of the first arch, laterally by the upper (*maxillary*) process of the first arch. Above it is bounded by a median plate, the *nasal process,* which on either side forms a protuberance, the *globular process.* Lateral to the globular process is a rounded depression, the *nasal pit.* The maxillary process extends forward and fuses with the globular process to form the upper jaw region (failure to unite resulting in the malformation known as 'hare-lip'). The nose is at first broad, due to the width of the nasal process, which later becomes the nasal septum (fig. 29). The nasal pits deepen and later acquire openings into the primitive mouth cavity.

FIG. 22.—HUMAN EMBRYO OF 4.2 MM., SHOWING THREE BRANCHIAL GROOVES. (After His.)



The viscera.—The structures so far considered belong, for the most part, to the body wall; it remains to consider the general plan of arrangement of the viscera. It has been pointed out that the body may be regarded as a cylinder enclosing two tubes, one of which constitutes the central nervous system and the other the digestive tract. The latter may be regarded as being primarily a straight tube traversing lengthwise the body cavity enclosed by the body wall (figs. 18, 20). The layers of both the visceral and somatic plates which immediately enclose the body cavity become transformed into a characteristic pleuro-peritoneal membrane. Near the mid-dorsal line, a vertical double plate of peritoneum extends ventrally connecting the somatic (parietal) and visceral layers of peritoneum, and constituting what is termed the *mesentery* (fig. 20).

Fig. 23.—Sagittal Section Showing the Primitive Pericardial and Cœlomic Communication, Septum Transversum, Liver, etc., in a Human Embryo of 3 mm. (After Kollmann, from a model by His.)

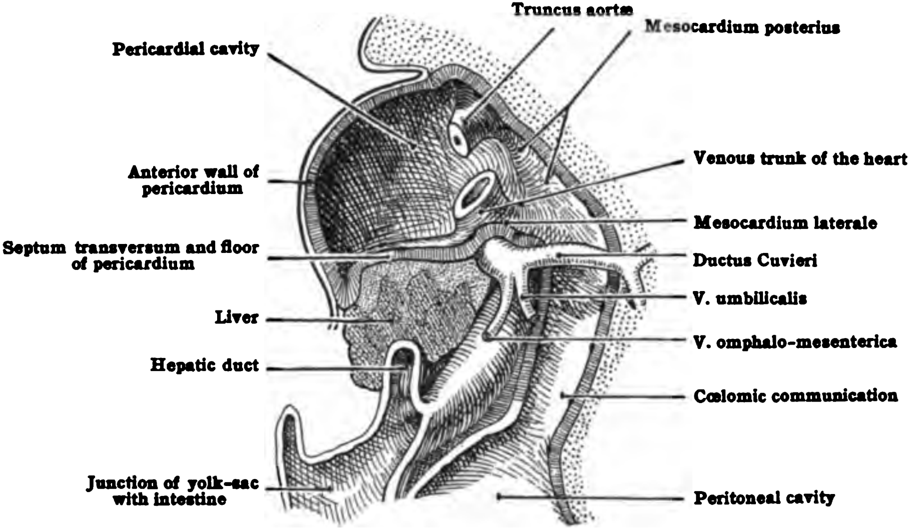
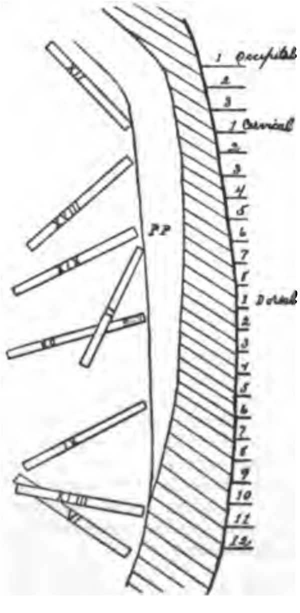


Fig. 24.—Diagram Illustrating the Recession of the Diaphragm (Septum Transversum) in the Human Embryo. On the right are indicated the vertebral levels; on the left, the position of the septum transversum in a series of embryos from 2 mm. (XII) to 24 mm. (VI) in length. *pp*, pleuro-peritoneal cavity. (Mall.)

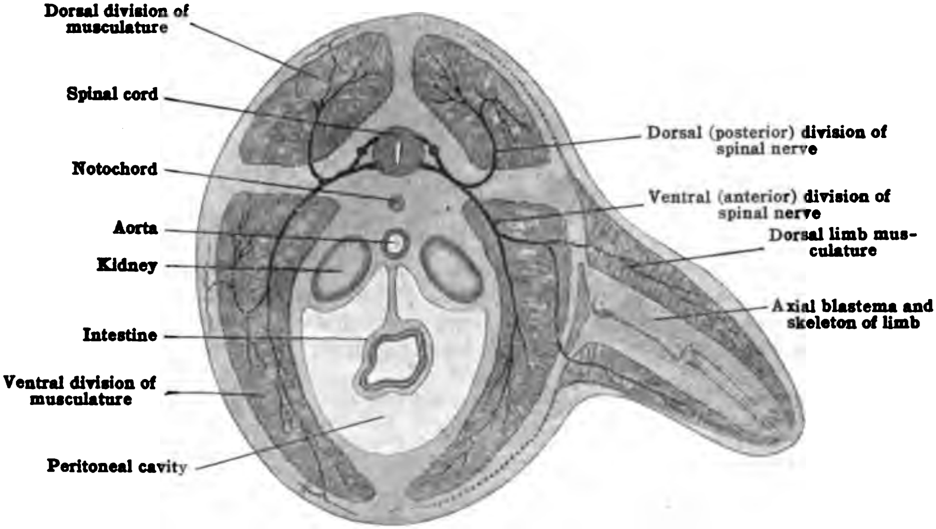


As development proceeds the digestive tract grows in length more rapidly than the cavity which contains it, and so gradually becomes thrown into numerous coils in the abdominal region, these changes leading to numerous modifications of the original arrangement of the mesentery. These will be described later on in the section on the digestive system.

the liver, the pancreas and the urinary bladder; and, with the exception of the bladder, each of these becomes completely invested by primitive peritoneum. In the case of the liver this original condition is practically retained, but the investment of the pancreas later becomes a partial one on account of the modifications which ensue in the mesentery. The bladder has only a portion of its surface in contact with the peritoneum, but the investment of the lungs remains complete, each lung, indeed, appropriating to itself the entire visceral layer of its half of the thorax, with the exception of a small ventral portion which forms the investment of the heart. Furthermore, the cavities which surround each of the three organs named, the two lungs and the heart, become completely separated from one another; and since each investment consists of a visceral and a parietal layer, each of the organs is enclosed within a double-walled sac, which in the case of each lung forms its *pleura*, while that of the heart is known as the *pericardium*. The spaces which occur within the thorax between the pleuræ of the two sides are known as the *mediastina*, which include the heart, œsophagus, etc. (fig 21).

In addition to the viscera mentioned there are some organs, such as the spleen and genito-urinary organs, which are developments of the mesoderm, the spleen arising in the mesentery which passes to the stomach and the genito-urinary organs primarily from the intermediate cell mass. The morphogeny of these structures and also of the vascular system, nervous system, and sense organs will be considered later in connection with their structure.

Fig. 25.—Diagram of a Cross Section of the Embryonic Body and Limb. (McMurrich, after Kollman.)


Dorsal division of musculature
Spinal cord
Notochord
Aorta
Kidney
Intestine
Ventral division of musculature
Peritoneal cavity
Dorsal (posterior) division of spinal nerve
Ventral (anterior) division of spinal nerve
Dorsal limb musculature
Axial blastema and skeleton of limb

Recession of the diaphragm and heart.—In the early stages of development the heart is situated far forward, in what will eventually be the pharyngeal region (figs. 12, 17). Just behind (caudal to) the heart, between it and the yolk-sac, is a plate of connective tissue, the *septum transversum*, which serves for the passage of large veins from the body wall to the heart (figs. 17, 23). This septum together with certain accessory structures eventually gives rise to the diaphragm, which becomes a complete partition separating the thoracic and abdominal portions of the body cavity.

The diaphragm and heart are therefore originally situated far above (cranial to) their final position and recede in the course of development, producing an elongation of the vessels and nerves associated with them and forcing downward such organs as the stomach and liver (fig. 24). The chief factor in this displacement is probably the ventral head flexion and the precocious growth and expansion of the organs in the head region. The effects of this recession are especially noticeable in the nerves, these passing to the various organs concerned arising from a much higher level than that occupied by the organs. The nerve to the diaphragm, for instance, comes from the fourth cervical segment, those passing to the cardiac and pulmonary plexuses from the cervical region, and those to the plexus in relation with the stomach, liver and adjacent organs from the thoracic region. The blood-vessels, however, may shift their origins from the main trunks by successive anastomotic roots, so that in general they keep pace with the viscera in the migration caudalward.

The limbs.—Each limb at its first appearance (fig. 22) is a flat, plate-like outgrowth from the side of the body, and consists of an axial mass (blastema) of mesodermic tissue from which the limb skeleton will develop, and, surrounding this, a layer, also of mesodermic tissue, from which the muscles and blood-vessels will arise. It is as yet uncertain whether the muscle blastema is derived from the

FIG. 26.—LATERAL VIEW OF A HUMAN EMBRYO 18 MM. LONG, SHOWING THE DEVELOPMENT OF THE EXTREMITIES. M, mandibular arch.

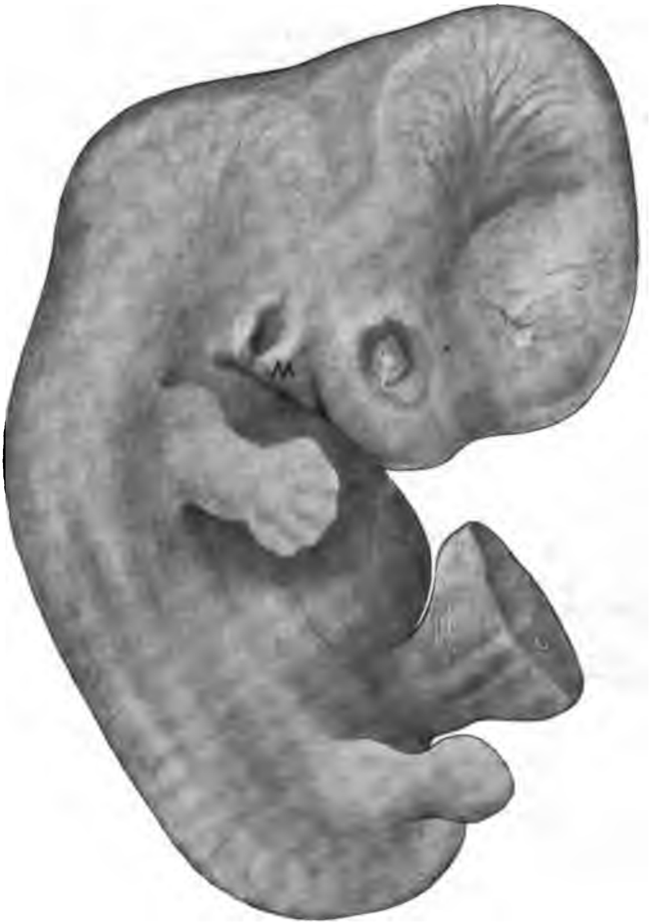
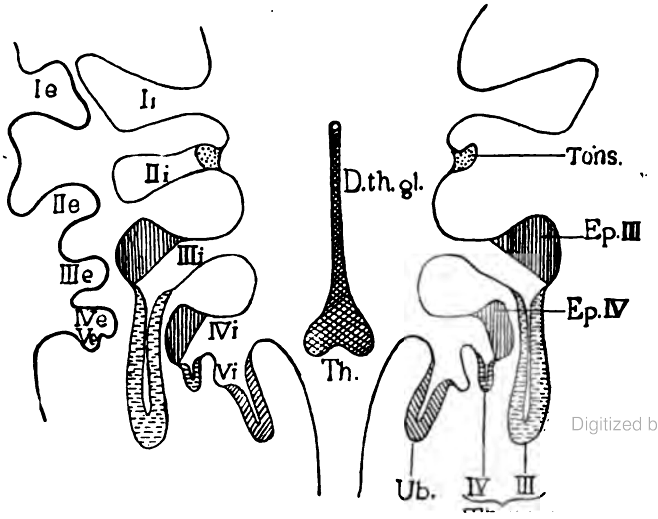


FIG. 27.—DIAGRAM TO SHOW THE DERIVATIVES OF THE BRANCHIAL CLEFTS. I*e*, II*e*, III*e*, IV*e*, V*e*, external branchial grooves. I*i*, II*i*, III*i*, IV*i*, V*i*, internal branchial grooves. Tons., palatine tonsil. *Ep III*, *Ep* IV, epithelial bodies. *Ub*, ultimobranchial body. *Th.*, thyreoid gland. *D.th. gl.*, ductus thyreoglossus. (Modified rom Keibel and Mall.)



As the muscles become differentiated, nerves grow to them from a definite number of spinal segments (fig. 25).

At first each limb plate is so placed that one of its surfaces looks dorsally and the other ventrally, and one border (that corresponding to the thumb or great toe) is anterior (i. e., cranial) and the other posterior (caudal). Later, however, each limb becomes bent caudally through about ninety degrees, so that the limbs whose long axes were at first at right angles to the long axis of the body come to lie parallel to that axis. In addition there occurs a rotation of each fore-limb in such a manner that the thumb turns latero-dorsally, while in the lower limb the direction of the movement is exactly the opposite, the great toe turning ventro-medially. As a result there is an apparent reversal of the surfaces in the two limbs, the flexor muscles of the arm reaching on the surface which is directed anteriorly, while in the lower limb the corresponding muscles occupy the posterior surface. The dorsum of the foot and the great toe side correspond respectively to the back and thumb side of the hand, the tibia corresponds to the radius and the fibula to the ulna. The limb anlage soon becomes divided into three primary segments. The distal segment (hand or foot) is a flattened rounded disc, in which the digits soon appear (fig. 26). The proximal portion forms the forearm or leg and the arm or thigh. In general, the extremities follow the law of cranio-caudal and dorso-ventral (proximo-distal) development.

FIG. 28A.—FACE OF HUMAN EMBRYO OF ABOUT 8 MM. (His.)

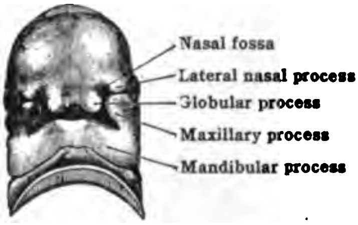
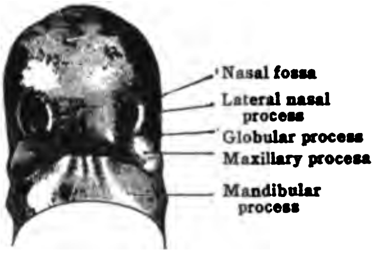


FIG. 28B.—FACE OF HUMAN EMBRYO AT STAGE SLIGHTLY LATER THAN 28A. (After Kallius.)



PRENATAL GROWTH IN LENGTH AND WEIGHT

Age in lunar months	Crown-rump or sitting height (Mall), cm.	Crown-heel or standing height (Mall), cm.	Weight at end of month, grams	Ratio of increase to weight at beginning of month
0	(diameter of ovum =0.2 mm.)	....	(Ovum = 0.000004 g.)	....
I	0.25	0.25	0.004	999.0
II	2.5	3.0	2.0	499.0
III	6.8	9.8	24.0	11.0
IV	12.1	18.0	120.0	4.0
V	16.7	25.0	330.0	1.75
VI	21.0	31.5	600.0	0.82
VII	24.5	37.1	1000.0	0.67
VIII	28.4	42.5	1600.0	0.60
IX	31.6	47.0	2400.0	0.50
*X	33.6	50.0	3200.0	0.33

Prenatal growth.—The prenatal growth of the human body in length and weight is indicated in the preceding table. According to Hasse, the age of the fœtus may be estimated from its total length as follows. Before the fifth month, the square of the age in (lunar) months gives the length in centimetres. After this, the age in months multiplied by five gives the length. This gives approximate results, except for the first month.

While the growth in *absolute* weight increases from month to month, it is important to note that the real (relative) growth rate rapidly diminishes. The ovum increases in weight during the first month about 1000 times, or 100,000 per cent. (not including the extra-embryonic structures). This rate diminishes rapidly, however, so that the increase during the last fœtal month is only about 33 per cent.

The continuation of growth in length and weight during the postnatal period is shown in the following chart (fig. 30).

The following chart is based upon data from Camerer (1–5 yrs.), Porter (6–17 yrs.), and Roberts (18–20 yrs.), showing the average postnatal growth in height and weight by sexes. The average height at birth is about 50 cm. (20 inches); weight, about 3200 g. (7 pounds). The male is slightly heavier and taller than the female, except during the acceleration at the period

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* 270 days (Mall).

FIG. 29.—FACE OF A HUMAN EMBRYO AFTER COMPLETION OF THE UPPER JAW. (McMurrich from His.)

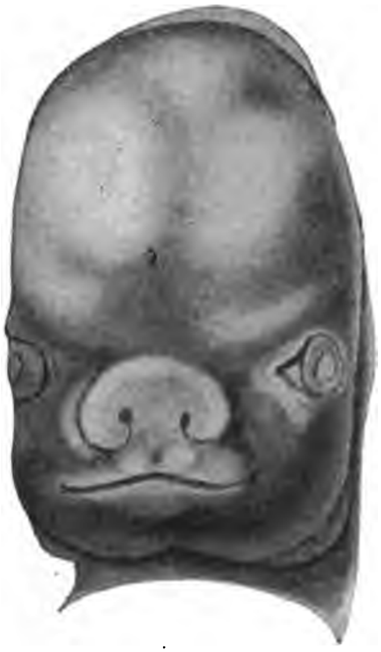
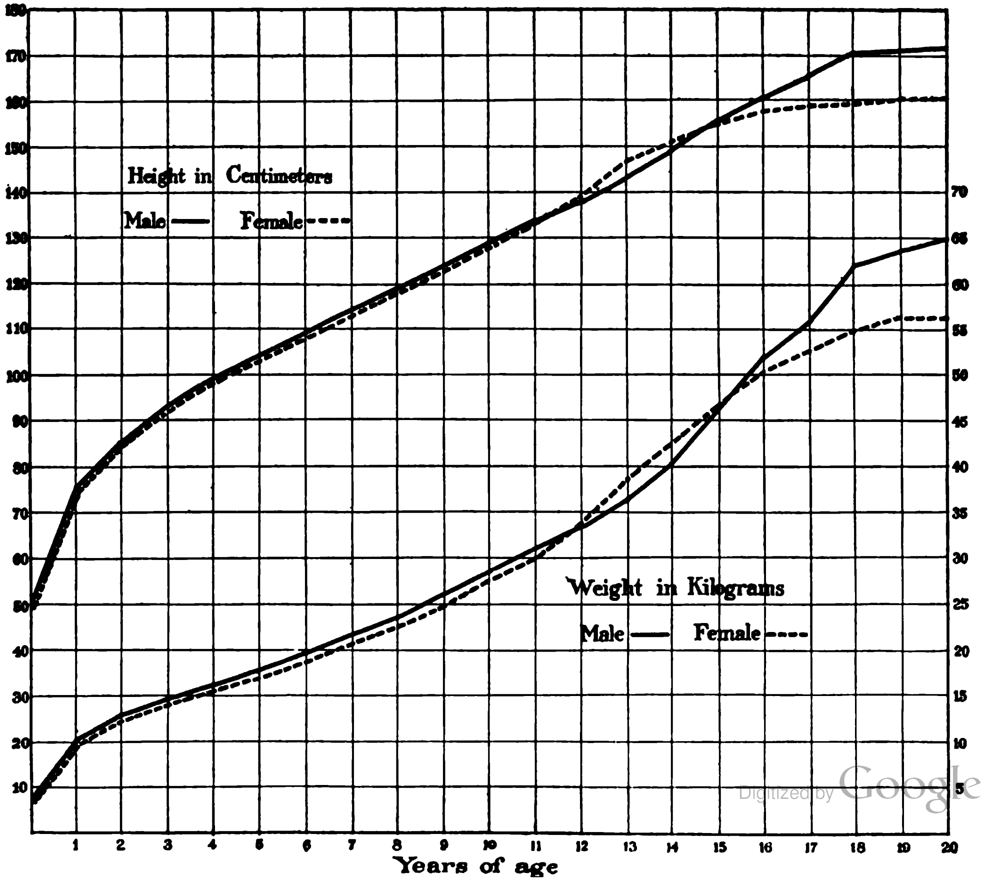


FIG. 30.—CHART SHOWING AVERAGE POSTNATAL GROWTH IN HEIGHT AND WEIGHT.



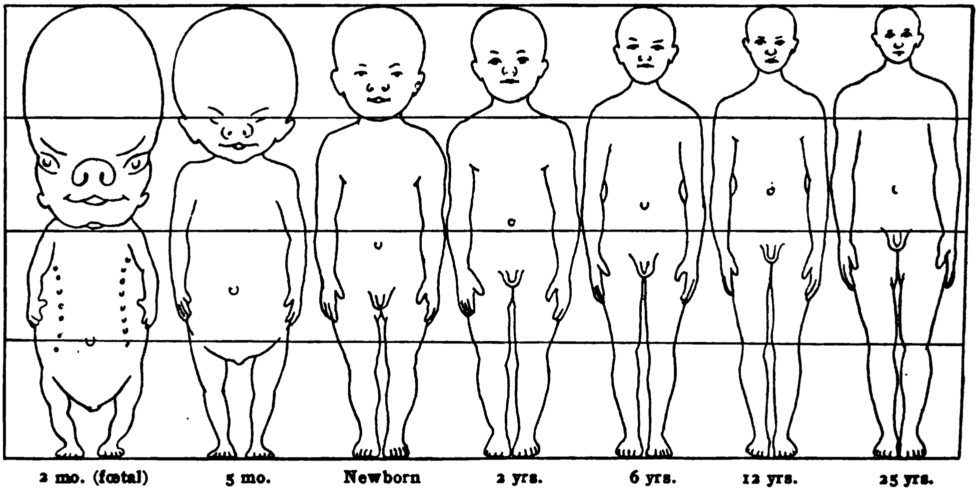
of puberty. Puberty occurs earlier in the female, so that between the ages of 12 and 15 the girls exceed the boys in average height and weight. With the exception of this period of acceleration, the (relative) growth rate in general diminishes steadily from birth, and has practically ceased at 20 years. The average height at this time is about 160 cm. (5 ft., 3 in.) in the female, and 170 cm. (5 ft., 7 in.) in the male; average weight, about 56 kilograms (126 lbs.) in the female, and 65 kilograms (146 lbs.) in the male. Under favourable conditions, growth in height may continue slowly up to about 25 years, and in weight even longer; but in old age there is a slight decrease in both height and weight.

The following measurements (from Holt, "Diseases of Infancy and Childhood" may be taken as a normal average standard of growth during the first three years. The weights are taken without clothing. The height is taken by placing the baby on a perfectly flat surface like a table, and having some one hold the child's knee down so that he lies out straight, then taking a tape-measure and measuring from the top of his head to the bottom of his foot, holding the tape line absolutely straight. The chest is measured by means of a tape line passed directly over the nipples around the child's body and midway between full inspiration and full expiration. The head measurement is taken directly around the circumference of the head, over the forehead and occipital bone.

		Weight, pounds	Height, inches	Chest, inches	Head, inches
Birth	Boys	7.55	20.6	13.4	13.9
	Girls	7.16	20.5	13.0	13.5
6 months....	Boys	16.0	25.4	16.5	17.0
	Girls	15.5	25.0	16.1	16.6
12 months...	Boys	20.5	29.0	18.0	18.0
	Girls	19.8	28.7	17.4	17.6
18 months...	Boys	22.8	30.0	18.5	18.5
	Girls	22.0	29.7	18.0	18.0
2 Years.....	Boys	26.5	32.5	19.0	18.9
	Girls	25.5	32.5	18.5	18.6
3 Years.....	Boys	31.2	35.0	20.1	19.3
	Girls	30.0	35.0	19.8	19.0

Relative growth of the parts.—The growth of the body is not uniform in the various parts, and changes in proportions therefore occur during development, as shown in fig. 31. It will be noted that the changes are in accordance with the law of developmental direction previously explained, the growth impulse passing along the body in a cranio-caudal direction.

FIG. 31.—FIGURES ILLUSTRATING THE CHANGES IN PROPORTION DURING PRENATAL AND POSTNATAL GROWTH. (STRATZ.)



The *head* is therefore largest in the earlier stages, forming about half the body, decreasing to 25 per cent. in the newborn, and to 7 or 8 per cent. of the body in the adult. The *upper limbs* increase to about 10 per cent. of the body at birth, maintaining thereafter about the same although the thoracic portion reaches its maximum in the earlier stages, and the pelvic portion not until adult life. The *lower limbs*, like the pelvis, develop slowly, forming about 20 per cent. of the body at birth and reaching 35 per cent. in the adult.

Relative growth of the systems.—There is also a marked difference in the relative growth of the various systems. Data for the *skin* and *skeleton* are somewhat scanty and unsatisfactory. The *musculature*, however, is relatively small in the embryo, increasing to about 25 per cent. of the body in the newborn, and to 40 or 45 per cent. in the adult. The visceral group (including brain and spinal cord), on the other hand, is relatively largest in the early embryo, decreasing from about 35 per cent. of the body to about 24 per cent. in the newborn and to about 10 per cent. in the adult.

Relative growth of the organs.—While in general, the individual organs follow the course of relative growth of the visceral group, each organ has its own characteristic course of growth. As a rule, after its appearance in the embryo, each organ increases more or less rapidly to its maximum relative size, after which, although increasing in *absolute* size, it decreases in *relative* size through subsequent prenatal and postnatal life up to the adult.

Thus the *brain* in the embryo of the second month forms more than 20 per cent. of the body, but steadily declines to about 13 or 14 per cent. in the newborn, and about 2 per cent. in the adult. The *spinal cord* and *eyeballs* have a similar course of growth. The *heart* declines from about 5 per cent. of the body in the embryo of the second month to about .75 per cent. in the newborn and .46 per cent. in the adult. The *liver* decreases from a maximum of nearly 10 per cent. in the third month to 5 per cent. in the newborn and 2.7 per cent in the adult. The *suprarenal glands* decrease from about .46 per cent. of the body in the third month to .23 per cent. in the newborn and .01 per cent. in the adult. The *lungs* decrease from 3.3 per cent. in the fourth month to about 2 per cent. of the body at birth and 1 per cent. (bloodless weight) in the adult. The *kidneys* reach a maximum of about 1 per cent. of the body toward the end of the fœtal period, decreasing to about .46 per cent. in the adult. The *thymus*, *thyroid*, *spleen* and *alimentary canal* likewise reach their maximum slowly, being probably relatively largest about the time of birth. The *ovary* and *testis*, however, appear to be relatively largest during the prenatal period.

Variability.—It must be borne in mind that all statements concerning structure refer to the *average* or *norm,* and are always subject to variation. This is therefore a topic of importance to students of anatomy. Variations are classified as either *germinal* or *somatic.*

Germinal variations are due to fundamental differences in the germ plasm, and are transmitted by heredity. These include many of the characters whereby one individual differs from another. Variations according to sex are included under this class. Variations inherited from more or less remote ancestors are termed *atavistic* or *reversional.*

Somatic variations, or 'acquired characters,' are due to environmental influences, such as nutrition, temperature, shelter, disease, training, etc. While somatic variations may be very great, they do not affect the germ plasm and are not transmitted by heredity.

In many cases it is exceedingly difficult to distinguish germinal from somatic variations Size, for example, may be due to either or both. Moreover, somatic variations may be produced at any time after the fertilisation of the ovum. Very slight environmental changes are sometimes sufficient to produce a marked effect upon the delicately balanced mechanism of the developing embryo. Malformations and pathological conditions are thus often to be explained. As to the *extent* of variability, some characters are much more variable than others. Height, for example, is less variable than weight. Moreover, variability differs in the various parts and organs. In general, the head and head organs are less variable than the remainder of the body. The skeleton and musculature appear less variable than the integument and viscera.

Details concerning variations and methods for their measurement may be found in works on genetics and biometrical statistics.

References.—*Embryology:* Keibel and Mall, Human Embryology (2 vols.); Bryce, Quain's Anatomy, 11th ed., vol. 1; Minot, Laboratory Text-book of Embryology; McMurrich, Development of the Human Body. *Growth:* Minot, Age, Growth, and Death; Jackson, Amer. Jour. Anat., vol. 9; Anat. Record, vol. 3. *Heredity:* Davenport, Heredity and Eugenics; Walter, Genetics. *Biometry:* Davenport, Statistical Methods; Yule, Theory of Statistics.

Reprinted from The Anatomical Record, Vol. 9, No. 1, 1915

27. Effect of acute and chronic inanition upon the relative weights of the various organs and systems of adult albino rats. C. M. Jackson, Institute of Anatomy, University of Minnesota, Minneapolis.

Twenty-one well-nourished adult rats were used, initial body weights varying from 182 to 367 grams. Fifteen rats were used for acute inanition, being allowed water but no food. They were killed after 6 to 12 days, the loss in body weight varying from 25 to 39 per cent (average loss, 36 per cent). Six rats were subjected to chronic inanition, being underfed so as to reduce the body weight slowly through a period of about five weeks, and were killed when the loss in body weight reached about 36 per cent. The results below stated apply in general to both acute and chronic inanition, unless otherwise specified. The published data of Donaldson, Hatai, Jackson and Lowrey are taken as normal for comparison. On account of the great variability of some organs and the relatively small number of observations, final conclusions are in some cases uncertain.

The head and fore-limbs lose relatively less than the body as a whole. Their relative (percentage) weight therefore increases. Of the systems —integument, skeleton, musculature, viscera and 'remainder'—the integument loses relatively nearly the same as the whole body, and therefore nearly maintains its original relative (percentage) weight. The same is true of the musculature, which however undergoes a somewhat greater loss in relative weight during chronic inanition. The visceral group, as a whole, undergoes little change in relative weight, decreasing slightly in acute inanition. The individual organs, however, vary greatly, as indicated below. The skeleton retains nearly its original absolute weight, and therefore increases greatly in relative weight. There is a corresponding decrease in the 'remainder,' due chiefly to loss of fat.

The individual viscera may be classified in three groups:

(1) The brain, spinal cord and eyeballs show little or no loss in absolute weight, compared with the normal at the initial body weight, hence their relative (percentage) weight has markedly increased with the diminution in body weight during inanition. The same is apparently true for the thyroid gland in acute inanition, but in chronic inanition there is apparently a loss, though relatively less than in the body as a whole. The suprarenal glands also apparently lose less in absolute weight than the body as a whole, hence their relative (percentage) weight increases.

(2) The heart, lungs, kidneys, testis, epididymis and hypophysis undergo nearly the same relative loss in weight as the body as a whole, therefore their relative (percentage) weight remains nearly the same. The thymus has already undergone age involution, and is therefore not materially affected by inanition.

(3) The liver and the alimentary canal (both empty and including contents) usually decrease in weight relatively more than the body as a whole. The spleen is exceedingly variable; in acute inanition it usually shows a marked decrease in relative weight (although averaging higher in chronic inanition).

Reprinted from The Anatomical Record, Vol. 9, No. 1, 1915

28. Changes in young albino rats held at constant body weight by underfeeding for various periods. C. M. Jackson, Institute of Anatomy, University of Minnesota, Minneapolis.

Ten litters, including 65 rats, were used. Twenty-five rats were used as controls, including 11 at 3 weeks of age, 2 at 6 weeks, 6 at 10 weeks, 3 at 32 weeks, and 3 at 35 weeks. In addition, data previously gathered by observations upon several hundred normal rats were available for comparison. Forty rats were held at constant body weight by underfeeding for various periods, 8 rats from age of 3 weeks to age of 6 weeks; 3 rats from 3 weeks to 8 weeks; 22 rats from 3 weeks to 10 weeks; 1 rat from 3 weeks to 13 weeks; 1 rat from 3 weeks to 16 weeks; 2 rats from 6 weeks to 32 weeks; and 3 rats from 10 weeks to 35 weeks. On account of the great variability of some organs, the number of observations in some cases is insufficient for final conclusions.

As to body proportions, the relative weights of the head, trunk and extremities remain practically unchanged in young albino rats held at constant body weights.

Of the systems—integument, skeleton, musculature, viscera and 'remainder'—there is but little change in the weight of the musculature, visceral group (as a whole) and 'remainder.' There is, however, usually a marked decrease in the integument, counter-balanced by a marked increase in the skeleton. Thus under these conditions the growth capacity appears weakest in the skin and strongest in the skeletal system. This is in striking contrast with the normal growth process, during which the musculature shows the greatest increase and the skeleton lags behind relatively.

The increase in the skeleton during constant body weight appears to involve the ligaments and cartilages as well as the bony skeleton. The skeletal growth tends to proceed along the lines of normal development, as indicated by changes in water content, by formation and union of epiphyses, and by relative elongation of the tail (compared with trunk length). The teeth also continue to develop normally (eruption of third molars).

The individual viscera may be classified in three groups.

(1) There is during the maintenance of constant body weight a well-marked increase in the weights of the spinal cord and eyeballs; usually also of the testis alimentary canal (both empty and including contents) and hypophysis.

(2) There is no marked change in the weights of the brain, heart, lungs, suprarenal glands, kidneys and epididymi. The liver is variable, with apparently a slight tendency to increase in the younger rats and to decrease in the older.

(3) There is always a marked decrease in the weight of the thymus ('hunger involution'); usually also of the spleen (earlier stages) and probably to a slight extent of the lungs and thyroid gland.

Reprinted from THE AMERICAN JOURNAL OF ANATOMY, Vol. 18, No. 1
July, 1915

EFFECTS OF ACUTE AND CHRONIC INANITION UPON THE RELATIVE WEIGHTS OF THE VARIOUS ORGANS AND SYSTEMS OF ADULT ALBINO RATS

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As emphasized by the author in a previous paper (Jackson '13), there is great need of a series of comprehensive growth norms, which would serve as a basis for experimental work and give a better insight into the growth process in animals. Through the investigations of Donaldson, Hatai, Jackson, Lowrey and others, considerable progress has been made toward the establishment of a growth norm for the albino rat. Since the normal growth process and the relative weights of the organs are frequently modified by malnutrition from various (sometimes unsuspected) causes, it is clearly of great importance to know the effects produced by inanition. In drawing conclusions from the results of any experiment upon animals, the possible effect of inanition should always be kept in mind. The present investigation upon this subject, which was begun at the University of Missouri, has been continued at the University of Minnesota with the aid of a special grant from the research fund of the Graduate School. The grant was used to employ a research assistant, who cared for the animals and assisted in the dissections, weighings, calculations, etc.

MATERIAL AND METHODS

The material included 21 well-nourished adult albino rats (Mus Norvegicus albinus) of unknown age. Two of these (O 5 and O 6) were derived from the Missouri colony and 19 from a local Minnesota stock. Of the 21, 4 were females and 17 males. They had been fed chiefly upon grain (oats and corn) with occasional meat and vegetables, and were placed upon a bread and milk diet for some days before beginning the experiment. During the experiment they were kept in cages with wire-net bottoms, allowing the feces (which might otherwise be eaten) to drop through.

Fifteen of the rats were used for the acute inanition experiment. They were allowed plenty of water, but no food otherwise. They were weighed daily and the individual records kept. The initial body weights varied from 182 to 367 grams (table 3). They were killed after 6 to 12 (average 9) days,[1] the total loss in body weight varying from 25 to 39 per cent (average loss, about 33 per cent). This represents moderately severe, but not extreme inanition, as a well-nourished adult albino rat will probably lose 40 per cent of his body weight before death, if kept warm. When pushed to the extreme, however, the rat is likely to die unexpectedly, with undesirable post mortem changes. These might affect not only the structure but also the weight of the organs, through congestion and coagulation of blood, and should therefore be avoided. Three of the rats included (table 3, rats Nos. M 9, M 13, and S 28) were found dead, and in this case merely the weights of the head, extremities, integument, skeleton and musculature (which did not appear to be materially affected) are recorded.

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[1] Bell ('11) found that the wild brown (Norway?) rat in captivity would live only 3 or 4 days, with loss of 25 or 30 per cent in body weight, when water is supplied but food withheld. Among animals in general, the older or larger are able to withstand inanition longer than the younger or smaller animals, probably on account of less rapid metabolism in the former.

In the rats subjected to acute inanition, the daily loss in body weight was fairly uniform in some individuals, but varied greatly in others. As might be expected, the loss is usually (though not invariably) greatest on the first day of the experiment, probably due to the reduction in contents of the stomach and intestines. In most cases (11 out of 15) the loss in body weight is greater during the first half than during the second half of the experiment, though the difference is usually slight. There is no constant relation between the percentage loss in body weight and either the initial body weight or the length of the inanition period. In general, the loss in body weight of albino rats during acute inanition is very similar in extent and variations to that found by Chossat ('43) for pigeons. Chossat demonstrated that the loss in body weight is greatest in the first third of the inanition period, slightly less in the last third, and considerably less in the middle third.

In order to compare the results of chronic inanition, 6 rats (all males) were fed upon a bread and milk diet gradually decreased in amount, so as to reduce the body weight about 1 per cent (of the initial weight) daily. Water *ad libitum* was also supplied. This was continued until the loss in body weight amounted to an average of about 36 per cent, which required about 5 weeks (table 3). The amount of food (entire wheat (Graham) bread soaked in whole milk), required for this purpose varied somewhat in individuals, but was approximately 10 per cent of the body weight daily. This is usually found insufficient for the maintenance of body weight in adult albino rats at ordinary room temperature. One rat (No. M 4) was found dead; the others were killed.

At the end of the experiment, in both acute and chronic inanition, the rats were killed by chloroform and the various organs and parts carefully dissected and weighed. The technique followed is that described by Jackson ('13) and Jackson and Lowrey ('12). The skeleton was first prepared as heretofore by carefully dissecting off the musculature, leaving the periosteum, ligaments and cartilages intact. This is recorded as 'ligamentous skeleton.' In most cases the periosteum and ligaments were then removed by maceration for about one hour in 1 per cent 'gold dust' solution, following which the bones were cleaned under water with forceps and camel's hair brush.[2] So far as possible, the cartilages, including the intervertebral disks, were left intact and included with the skeleton. The skeleton was then taken from the water (excess moisture removed by filter paper) and weighed as 'cartilaginous skeleton.' The skeleton was then dried for 30 days in a dust-proof case at ordinary room temperature. This was found insufficient to remove all moisture, so the skeletons were finally dried several days in an oven at about 90°C., until a constant weight was reached.

Portions of the various tissues and organs were preserved for histological study, which will be considered in a later paper.

As heretofore, in calculating the percentage weights, the net body weight (gross body weight less intestinal contents) is taken. The percentage weights of the organs are therefore slightly higher than they would be if calculated upon the basis of the gross body weight, but the difference is usually negligible, as the intestinal contents form only 2 or 3 per cent of the body weight during inanition.

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[2] For this method of preparing the cartilaginous skeleton, I am indebted to Professor Donaldson of The Wistar Institute. He states (in a personal communication) that the fresh skeleton is apparently slightly reduced (about 3 per cent) in weight by the 'gold dust' treatment, but this difference is so small as to be practically negligible.

For purposes of comparison, the previous observations upon the normal weights of the various organs of the albino rat by Donaldson ('09), Hatai ('13), Jackson ('13) and Jackson and Lowrey ('12) were utilized. Some unpublished data from the Missouri Agricultural Experiment Station upon a series of six steers, varying from very fat to thin, are cited through the courtesy of Professors Trowbridge and Moulton.

The averages given in table 3 are the arithmetical means of the corresponding individual observations in the acute and the chronic inanition series, respectively. In estimating the normals at corresponding initial body weights for comparison, however, merely the averages were used. That is, the absolute weight of each organ corresponding to the *average* body weight was estimated (from data already available for the normal rat), and the corresponding percentage weight calculated. This is not quite so accurate as it would have been if the corresponding normal for each individual had been estimated, and the mean of these taken for comparison with the averages in the acute and chronic inanition series. However, the difference is slight and apparently not sufficient in the present series to justify the more laborious method of making the individual estimates.

In view of the comparatively small number of observations, and the known variability, especially of some of the organs (Jackson '13), the conclusions reached in the present paper are by no means to be considered as final. It is believed, however, that they are sufficient to give an approximate idea of some of the more obvious and important changes in weight during inanition. As such they may be useful, even though limited in number, and may lead to further and more extensive investigations in the case of various individual organs. In general, the amount of variation found is sufficient to demand the exercise of caution in drawing conclusions from an insufficient number of observations, as is sometimes done in experimental work.

Although the literature on the subject of inanition is extremely large, comparatively few specific data directly bearing upon the question of the changes in the weight of the various organs are to be found. These are referred to later under the appropriate headings. Papers dealing merely with histological changes are usually not mentioned. Extensive references to the literature on inanition will be found in the articles by Morgulis ('11) and Mühlmann ('99).

LENGTH OF BODY AND TAIL

With reference to the body weight compared to the body (nose-anus) length at the end of the inanition period, the normal relations as determined by Donaldson ('09) were used for comparison. As might be expected, the body weight following inanition, both acute and chronic, is found to be lower than normal for corresponding trunk length, since the skeleton is known to be in general but slightly affected by inanition. The difference, however, is surprisingly small. It is a noteworthy fact that the *initial* body weight of the animals used in he experiments is far greater than that normal for the body length at the *end* of the inanition period. (The length cannot be accurately measured in the living animal without anesthetics at the *beginning* of the experiment.)

TABLE 1

	ACUTE INANITION SERIES: GRAMS	CHRONIC INANITION SERIES: GRAMS
Normal gross body weight (theoretical, Donaldson) corresponding to body length at end of inanition period, average (from individual calculations)	191	147
Actual gross body weight found at beginning of the inanition period, average (table 1)	255	214
Actual gross body weight found at end of the inanition period, average	170	136

Thus, as shown in table 1, the body length at the end of the inanition period corresponds to a body weight much nearer to the final than to the initial weight of the animals subjected to inanition.

There are two possible explanations for this surprisingly close approach to the normal in the body weight after inanition. It is possible that the rats at the beginning of the experiment were somewhat too heavy for the normal at corresponding body length. This might account for the result, even on the assumption that the body length has remained constant during the period of inanition. On the other hand, it is more probable that there has been an actual slight decrease in the trunk length during inanition (probably due to shrinkage of the intervertebral disks), which would equally well account for the facts observed. Individual variations must also be kept in mind, as well as the possibility that the normal ratio of body weight to body length in the strain of rats used may differ somewhat from the normal, as determined by Donaldson.

The theory of a decrease in the trunk length during inanition is strengthened by the apparently changed ratio of tail length to body length. In a separate paper (Jackson '15) I have shown that the normal ratio of the tail length to body length in the albino rat increases from an average of about 0.36 in the newborn to 0.88 at 6 weeks (body weight 50 grams), decreasing slowly to about 0.85 at a body weight of 200 grams and to about 0.80 at 300 grams. (The lower ratio found in the heavier rats is partly due to the absence of females, in which the tail averages relatively longer than in the males.) Thus it appears that in rats with body weights corresponding to the initial weights in the inanition series, the normal average ratio of tail length to body length should be between 0.80 and 0.85. It is impracticable to make the actual measurements on the living animals, although this might be done by the use of anesthetics. The data given in table 3 show that at the end of the inanition period, however, the average ratio in the acute inanition series is 0.93, and in the chronic inanition series, 0.97. Thus it appears that inanition in adult albino rats tends to produce relatively long-tailed individuals, due probably to a shrinkage in the trunk length.

Hatai ('08) reached the opposite conclusion, viz., that underfeeding produces *short-tailed* individuals; but his observations were upon younger, growing rats, in which the conditions might be somewhat different. Morgulis ('11) in the salamander, Diemyctylus, likewise found a greater shrinkage in the tail during inanition, while Harms ('09) found the converse to be true in

Triton. This question is discussed more fully in the paper above referred to (Jackson '15), the conclusion being that inanition in young rats also tends to produce relatively long-tailed individuals.

HEAD

The head (fig. 1; table 3) in acute inanition averages about 11.2 per cent of the body weight, varying from 10 per cent in the larger rats to 13.9 per cent in the smaller. Normally (Jackson '13; Jackson and Lowrey '12) in rats corresponding to the body weight at the beginning of the experiment, the head should range from about 8 per cent in the larger rats to 10 per cent in the smaller, the average for the group being about 9 per cent. Thus we may assume that during the period of acute inanition the head has increased from an average of about 9 per cent to about 11.2 per cent of the body. This is an increase of about one-fourth in the relative size of the head.

Since the whole body has lost an average of one-third in absolute weight, it might appear at first glance that the head has remained nearly constant in absolute weight. This, however, is not true. If the body weight decreased one-third while the head remained constant in absolute weight, the *relative* (percentage) weight of the head would increase in the ratio of 2:3 or from 9 to 13½ per cent. A study of the absolute weights of the head, compared with the normal, at the beginning of the experiment shows that the head has actually lost weight, but in much smaller proportion than the body as a whole. This is what is to be expected since, as will be shown later, the brain, eyeballs and skeleton in general lose but little or none in absolute weight during inanition; while the loss in fat (some of which is on the head) is great, and the loss in the integument and musculature is in nearly the same proportion as in the body as a whole.

The head of the 6 rats subjected to chronic inanition averages 11.4 per cent of the body weight, slightly higher than in acute inanition. The rats used in chronic inanition averaged smaller in body weight (table 3), and the normal relative initial weight of their heads should therefore be slightly higher, about 10 per cent (instead of 9 per cent, as in the acute inanition group).

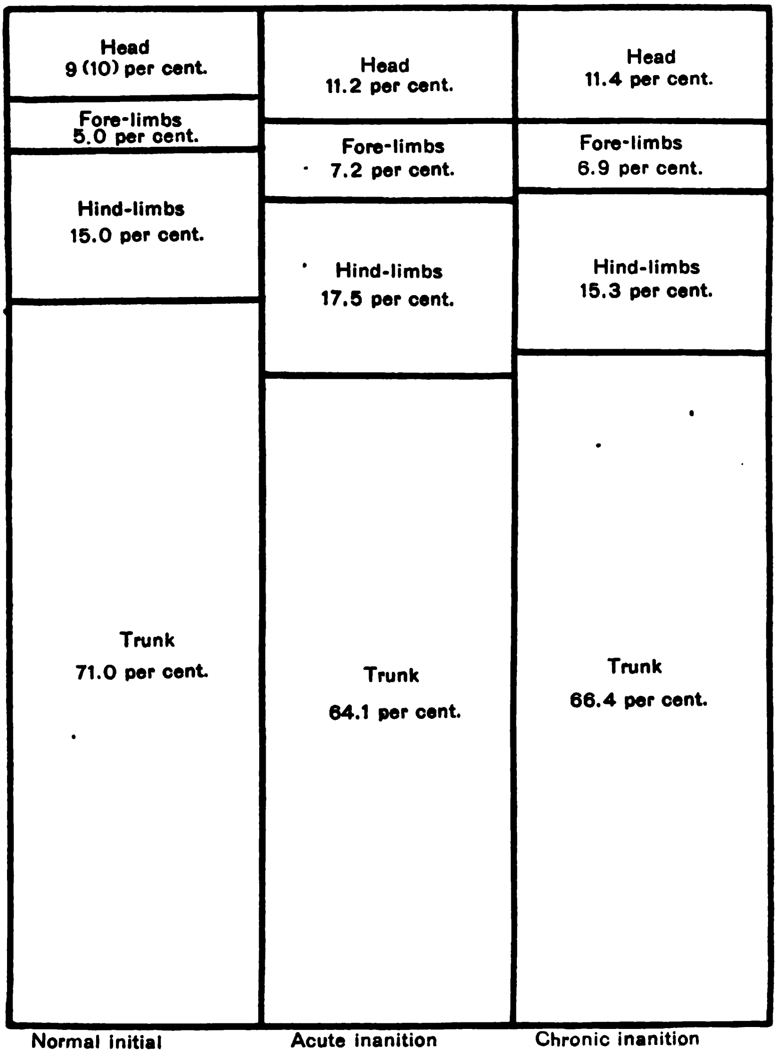

Fig. 1 Diagram representing the relative (percentage) weights of the head, trunk and extremities of adult albino rats. The first column represents the relations found in normal rats corresponding to the average initial body weight of those used in the experiments. The second column represents the relations found in the acute inanition series, and the third column those found in the chronic inanition series.

EXTREMITIES AND TRUNK

The extremities were separated from the trunk at the shoulder and hip-joints, respectively. From figure 1 and table 3 it is seen that the *fore limbs* formed an average of 7.2 per cent of the body in the acute inanition series, and 6.9 per cent in the chronic inanition series. According to Jackson and Lowrey ('12), in the normal adult rat the fore limbs form about 5 per cent of the body. Consequently it would appear that there has been a relative increase in the weight of the fore limbs during inanition, especially during acute inanition. This increase is greater than would be expected, since (as will appear later) the skeleton is the only important constituent of the limbs which increases in relative weight during inanition, the integument and musculature remaining relatively constant. The conclusion as to the fore-limbs should be regarded as uncertain, however, because of: (1) the difficulty in separating the limbs (especially the skin) in a uniform manner; (2) the comparatively small number of observations upon which both the normal and the experimental average is based; (3) the apparent lack of agreement between the results for the entire fore limbs and for their components, skin, skeleton and musculature. It is possible, on the other hand, that the losses for skin, skeleton and musculature are not uniform in all parts of the body. Chossat ('13) has shown, for example, that the great pectoral muscles in pigeons lose relatively much more than the remainder of the musculature during inanition.

The *hind limbs* (fig. 1; table 3) in the normal adult form about 15 per cent of the body (Jackson and Lowrey). As might be expected, this is slightly increased, to 17.5 per cent, in the acute inanition series, probably on account of the relatively heavier skeleton. In the chronic inanition series, the hind limbs average 15.3 per cent, or about the same as normal. This may be explained as due to the relatively greater loss in the integument and musculature during chronic inanition (as will be shown later), this loss tending to counterbalance the relative increase in the skeleton.

The *trunk* (fig. 1; table 3) is measured by substracting the weight of the head and extremities from the net body weight.

It forms normally about 0.71 per cent of the adult body (Jackson and Lowrey). During inanition, the trunk becomes relatively smaller, averaging 64.6 per cent in the acute inanition series (64.1 per cent, corresponding to the average of 11.2 per cent for the head in a larger series), and 66.4 per cent in the chronic inanition series. The relative decrease in the trunk of course counterbalances the relative increase in the head and extremities.

INTEGUMENT

As shown in figure 2 and table 3, the integument (which includes the skin and appendages, hair and claws) is fairly uniform in its relative weight, averaging 18.9 per cent in acute inanition (or 19.1 per cent in those cases in which the viscera were weighed), and 17.8 per cent in chronic inanition. The average integument for normal adults (fig. 2) corresponding to the initial size of these rats forms about 18 per cent of the body weight (Jackson and Lowrey '12). It is therefore evident that during both acute and chronic inanition in adult albino rats the loss in weight of the integument is nearly proportional to that of the body as a whole, so the relative (percentage) weight remains almost the same.

In absolute weight, the integument has apparently decreased from about 45.9 grams, the normal at the average initial body weight in the acute inanition series (255 grams), to an average of 31.6 grams, as shown in table 3. This would correspond to a loss of 31.2 per cent in the weight of the integument. In the chronic inanition series, the corresponding decrease is from 38.5 to 23.7 grams, a loss of 38.4 per cent. Apparently, therefore, the loss in the weight of the integument is relatively slightly greater during chronic than during acute inanition.

The data found in the literature concerning the relative loss of the integument during inanition are not in agreement. In the dog, Aron ('11) states that the skin loses relatively more than the muscles (therefore, more than the body as a whole); Voit ('05 b) cites data showing a relative increase in the (fat-free) skin; while Falck's ('54) data show the skin relatively unchanged in weight. In the rabbit and cat, observations by Pfeiffer ('87), Voit ('66)

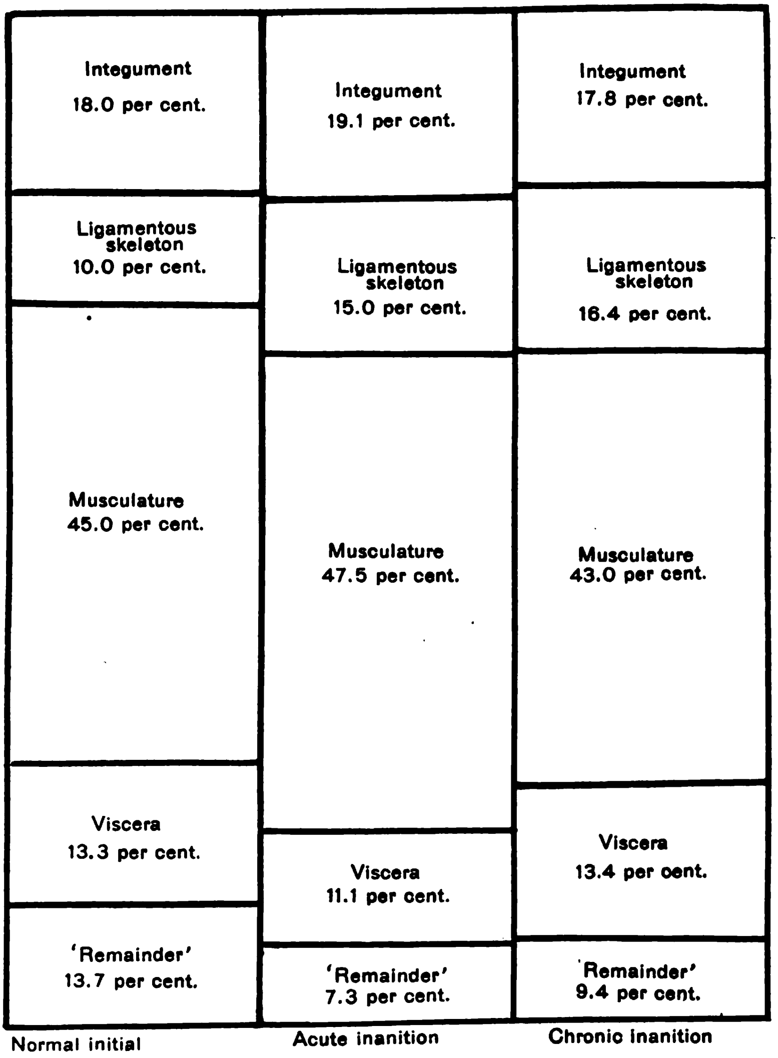

Fig 2 Diagram representing the relative (percentage) weights of the various systems (integument, skeleton, musculature, viscera and 'remainder') of adult albino rats. The first column represents the relations found in normal rats corresponding to the average initial body weight of those used in the experiments. The second column represents the relations found in the acute inanition series, and the third column those found in the chronic inanition series.

and Sedlmair ('99) show a relative increase in the skin during inanition. Much of the variation is doubtless due to the differences in the relative amount of fat present in the integument of the normal animal. The subcutaneous fat is not abundant in the rat. Some unpublished data from the Missouri Agricultural Experiment Station upon a series of steers show that the percentage of the hide increases from 5.5 per cent in a very fat animal to 8.5 per cent of the body in a thin animal.

SKELETON

The ligamentous skeleton (table 3; fig. 2) forms an average of 15.3 per cent of the body after acute inanition (15.0 per cent in the series in which the viscera were weighed), and 16.4 per cent after chronic inanition. Since the average for the normal rat corresponding to the initial body weight of the animals used is only about 10 per cent of the body weight (Jackson and Lowrey '12), it is evident that while the body weight has decreased one-third during the period of inanition, the ligamentous skeleton has decreased little or none in absolute weight. If the normal skeleton formed 10 per cent of the body, and remained constant while the body lost one-third in weight, the *relative* weight of the skeleton would be increased one-half, or to 15.0 per cent. This corresponds with the observations as closely as could be expected.

In terms of absolute weight, assuming that the skeleton formed 10 per cent of the normal initial weight, the skeleton apparently decreased from 25.5 to 25.4 grams in the acute inanition series (loss of 0.4 per cent); and *increased* from 21.4 to 21.8 grams in the chronic inanition series (*gain* of 1.8 per cent). No great stress can be laid upon the accuracy of these figures, however, on account of the small number of observations, and the variability and uncertainty as to the normal weight.

The cartilaginous skeleton, including the bones and cartilages after removal of the ligaments by maceration (as previously described), forms an average of 10.9 per cent of the body in seven cases of acute inanition, and 12.4 per cent in the chronic inanition series (table 3). No published observations upon the normal cartilaginous skeleton are available for comparison. Professor Donaldson of The Wistar Institute, however, has very kindly sent me a series of observations upon the weight of the cartilaginous skeleton in normal albino rats. The average, in 10 cases with body weight varying from 194 to 426 grams, forms about 6.7 per cent of the (gross) body weight. In this series, however, I understand that the intervertebral cartilages were not preserved, so this figure is probably somewhat too low for use as a normal to be directly compared with my observations upon the cartilaginous skeleton in inanition. The normal adult cartilaginous skeleton, including the intervertebral cartilages, would *probably* form about 7 per cent (or slightly more) of the net body weight.

The dried cartilaginous skeleton (table 3) formed an average of 5.48 per cent of the net body weight in 7 cases of acute inanition, and 6.03 per cent in the 6 chronic cases. Thus it is evident that in each series the cartilaginous skeleton is composed of approximately half dry substances and half water. In the acute inanition series, the dry substance in the cartilaginous skeleton averages 50.5 per cent (range, 45.1 to 54.6 per cent). In the chronic series, the dry substance averages 49.2 per cent (range, 45.5 to 55.7 per cent). If case M 5 be excluded (which appears to be exceptional or erroneous), the average for the chronic series would be still lower, or 47.9 per cent. In any event, the dry content appears to be slightly lower and the water content correspondingly higher on the average in the chronic inanition series, which might be expected.

Data upon the normal composition of the skeleton of the rat for comparison are scarce. In one case which I have observed (at 10 weeks) the net body weight was 115 grams, ligamentous skeleton 9.91 per cent of body weight, fresh cartilaginous skeleton 5.57 per cent, and dry cartilaginous skeleton 2.97 per cent. In this case the dry substance formed 53.4 per cent of the cartilaginous skeleton. Lowrey ('13) in two albino rats (body weight 267.5 grams) finds the *ligamentous* skeleton forming about 9 per cent of the body weight, and containing 52.6 (52.1 to 53.1) per cent of dry substance. (This would probably be slightly higher than the dry content of the *cartilaginous* skeleton, however, on account of the higher percentage of water in the ligaments.) Further data are necessary before final conclusions can be reached, but it appears that in the rat (as in other animals) the percentage of water in the skeleton is increased during inanition.

It is a well known fact that in general the skeleton loses comparatively little during inanition, and thus increases greatly in relative (percentage) weight. In a series of six steers, the skeleton varied from 10.6 per cent of the body in a very fat animal to 19.3 per cent in a thin animal. (Data from the Missouri Agricultural Experiment Station.) An apparent slight loss in absolute weight of the (cartilaginous?) skeleton has been observed during inanition as follows: pigeons, by Chossat ('43), 3 per cent; cats, by Voit ('66), 14 per cent; little or none by Sedlmair ('99); dogs, little or none by Voit ('05 a) and Falck ('54); rabbits, little or none by Gusmitta ('84), Weiske ('95) and Pfeiffer ('87).

Since the calcified framework is but little affected in volume during inanition, it is evident that there can be but little, if any, loss in absolute weight of the skeleton. It is at least theoretically possible that there may even be a slight *increase* in its weight, since the fat in the bone-marrow is replaced by a mucoid substance (Jackson '04) presumably of higher specific gravity. Examination of bones, therefore, invariably shows a marked increase in water content during inanition. As Sedlmair ('99) states (p. 33): "Es giebt kein einziges sicheres Beispiel für einen geringeren Wassergehalt der Knochen hungernder Tiere; alle Forscher (Chossat und Lukjanow an Tauben, Gusmitta, C. Voit und Schöndorff an Hunden, Weiske an Kaninchen) fanden darin einen höheren Wassergehalt." This would naturally vary in different bones, and in different animals, according to the relative fat content. Small animals, like the rat, have in general relatively much less marrow fat than larger animals.

MUSCULATURE

The musculature (table 3; fig. 2) forms an average of 47.6 per cent of the body in the acute inanition series, which is slightly higher than the average for the normal adult rat (which is about 45 per cent, Jackson and Lowrey '12). The musculature in the chronic inanition series is somewhat lower, the average being 43.0 per cent of the body weight. This would indicate a somewhat greater loss relatively in chronic inanition.

In terms of absolute weight, the musculature would appear to have decreased from 114.75 to 79.25 grams (a loss of 30.9 per cent) in the acute inanition series; and from 96.30 to 57.0 grams (a loss of 40.8 per cent) in the chronic inanition series.

There is much variation in the weight of the musculature following inanition, judging from data available in the literature. The statements usually refer to acute inanition. According to Gaglio ('84), while the body weight of the frog loses 56 per cent the musculature loses 85 per cent of its weight. The musculature appears to suffer a slight relative loss (decrease in percentage weight) in dogs (Falck '54; Voit '05 b) and cats (Sedlmair '99), while it remains nearly unchanged in the rabbit (Pfeiffer '87; Voit, '05 b). On the other hand, data from a series of steers slaughtered in the Missouri Agricultural Experiment Station indicate a relative increase in the musculature from about 33 per cent of the body weight in a very fat animal, to 44 per cent in a lean animal, due probably to the earlier loss of the fat. Voit ('66) finds a slight relative increase in the musculature of the cat. Lasarew ('97) in an extensive series of guinea-pigs subjected to various degrees of inanition found that the loss of the musculature is somewhat less than that of the body fat in the earlier periods, but greater in the later periods. While the body weight lost 10 per cent, the musculature lost only 7.28 per cent of its weight; but the loss of the musculature was much greater at later periods. This would perhaps explain the relatively greater loss of the musculature during chronic inanition.

VISCERA AND REMAINDER

The average for the total visceral group in the normal adult rat, according to Jackson and Lowrey ('12; based upon only a few observations) is about 13.3 per cent of the body weight. In the acute nanition series (table 3; fig. 2) the average found is 11.1 per cent; while n the chronic series it is 13.4 per cent. This would seem to indicate that the loss in weight is relatively greater in acute than in chronic inanition. As a matter of fact, the great majority of the individual organs, as will be seen later, show a greater relative loss during chronic inanition. The liver and spleen are exceptions, however, and the large bulk of the former overbalances the other viscera when all are grouped together. On the whole, there is not much change in the relative weight of the visceral group; but there is, however, much variation among the individual organs, as will be seen later.

Aron ('11) states that the organs lose more than the musculature during inanition in dogs. Data by Voit ('05 b) show a decrease in the relative size of the viscera in the rabbit, but not much change in the dog.

The 'remainder' is the amount obtained by substracting from the net body weight the weight of the skin, skeleton, musculature and visceral group. It includes the loss by evaporation and escape of liquids, a few small unweighed organs, and the dissectable masses of fat. In the normal adult rat, the 'remainder' forms about 13 per cent of the body weight (Jackson and Lowrey '12). In the chronic inanition series, the average is 9.4 per cent. In the acute series the average is 7.3 per cent. The decrease in the relative weight of the 'remainder' is probably due chiefly to loss of fat.

BRAIN

The average for the brain in the acute inanition series (table 1) is 1.18 per cent of the body weight. By means of the tables and curves constructed by Donaldson ('09), the (theoretical) normal weight of the brain can be derived, corresponding to any given body weight. This gives an average of 0.78 per cent for the assumed normal weight of the brain at the beginning of the experiment (gross body weight, 244 grams). Since the average loss in body weight during the inanition period is one-third, it is evident that the brain has lost but little if any in absolute weight.

The theoretical average initial absolute weight of the brain is about 1.902 grams, forming 0.78 per cent of the (gross) body weight (Donaldson '09). According to Donaldson's ('08) table 1, the brain weight observed in six cases averaged 1.900 grams, the theoretical being 1.905 grams, at the body weight of 245 grams. If this absolute weight of the brain remained constant, while the body weight lost one-third (33.9 per cent), the final percentage weight of the brain would be increased about one-half, or to 1.17 per cent. The average absolute weight of the brain actually observed at the end of the period of acute inanition is 1.8046 grams, corresponding to an average of 1.18 per cent of the (net) body weight.[3] This weight is slightly less than would be expected if the brain remained constant in absolute weight, indicating a loss of about 5.1 per cent in the absolute weight of the brain during inanition.

The observations upon the brain in chronic inanition (table 3) indicate a similar condition. The average brain in this series weighs 1.743 grams and forms an average of 1.33 per cent of the (net) body weight. (This is the mean of the individual percentages; 1.743 grams would form 1.31 per cent of the average net body weight, 132.5 grams; or 1.28 per cent of the average corresponding gross body weight, 136 grams.) According to calculations from Donaldson's ('09) data, the average brain corresponding to the body weight at the beginning of the experiment (213.7 grams) should weigh 1.866 grams, forming about 0.87 per cent of the body weight (the percentage being higher than in the acute inanition series on account of the smaller average body weight).[4] This is slightly higher than the result to be expected on the assumption that the absolute brain weight has remained constant, while the body weight has decreased about one-third (36.1 per cent). In other words, the results indicate a loss of about 6.6 per cent in the absolute weight of the brain during chronic inanition, which is slightly greater than the apparent loss (5.1 per cent) during acute inanition. A larger series of observations would be necessary, however, to draw any final conclusions with precision.

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[3] This is the mean of the individual percentages, as shown in table 3. The average brain weight, 1.8046 grams, would correspond to 1.13 per cent of the corresponding average gross body weight, 160 grams, at the end of the experiment.

[4] According to Donaldson's ('08) table 1, corresponding to a body weight of 215 grams, the average of 8 brains, both sexes, was 1.873 grams, the corresponding theoretical weight by formula being 1.871 grams.

The data agree fairly well with the results of Hatai ('04) who found an estimated loss of about 5 per cent in the weight of the brain of young rats during chronic inanition. Donaldson ('11), however, found in still younger rats an actual *increase* of 3.6 per cent in the brain weight during chronic inanition (body weight held constant from age of 30 days to 51 days); while my own observations (Jackson '15) indicate little or no change in the brain weight under these conditions.

It has long been known that of all the organs of the body, the central nervous system apparently suffers least in weight (if at all) during inanition. Thus Chossat ('43) found no loss[5] in the weight of the brain in starved pigeons, with loss in body weight of about 40 per cent. Falck ('54) in dogs, and Lasarew ('97) in guinea-pigs, found little or no loss in the weight of the brain and cord during inanition. Bowin ('80) and Pfeiffer ('87) found an absolute decrease (but relative increase) in the brain weight of the rabbit. Voit ('66) in cats found a decrease of about 3 per cent in the weight of brain and cord; while the data of Sedlmair ('99) would even indicate an actual *increase* in their weight during starvation. Data from a series of steers slaughtered in the Missouri Agricultural Experiment Station show that the absolute weight of the brain and cord in thin animals is but very slightly less than in very fat animals with nearly double the body weight. The relative (percentage) weight is of course correspondingly higher in the thin animals.

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[5] Since Chossat's results are often misquoted, it may be noted that he found the average weight of the brain almost identical in the 8 starved pigeons and in 8 controls of nearly the same (initial) body weight; (brain weight average 2.27 grams for starved; 2.25 grams for corresponding controls). On account of the difficulty in determining the exact plane of separation between the brain and spinal cord, however, Chossat preferred to combine their weights, thus giving a slight decrease (about 1.9 per cent) in the absolute weight of the entire central nervous system during inanition.

In young animals during the period of active growth, the results of inanition (especially chronic inanition) are in general somewhat different from those in adults, since the tendency of the organs to maintenance is in the young animal complicated by the growth impulse (Jackson '15). Bechterew ('95) found a slight apparent loss in the absolute weight of the brain and spinal cord of the newborn cat and dog during acute inanition. Hatai ('04) found in a series of young albino rats subjected to chronic inanition for 21 days an estimated average loss of about 5 per cent in the absolute weight of the brain. Later (Hatai '08) in another group of young rats in which growth had been retarded by underfeeding, he found that in these stunted animals the brain and cord had a weight approximately normal for the corresponding body weight. Donaldson ('11), in still younger rats held at nearly constant body weight by underfeeding from age of 30 days to 51 days, finds an apparent *increase* of 3.6 per cent in the weight of the brain. My own observations (Jackson '15), however, indicate little or no change in the weight of the brain under these conditions.

On the whole it appears that in adults inanition, both acute and chronic, produces a slight loss in the absolute weight of the brain. In young, rapidly growing animals, however, it is doubtful whether any such loss occurs, especially in chronic inanition, where there is even a possible increase in brain weight.

SPINAL CORD

The spinal cord in the acute inanition series (table 3) has an average weight of 0.631 gram and forms an average of 0.41 per cent of the body weight. Calculations from Donaldson's ('09) data indicate that at the average initial gross body weight (244 grams) the spinal cord should weight 0.625 gram, forming 0.26 per cent of the body weight. The data would therefore seem to indicate not only no loss, but even a very slight *gain* of the spinal cord in absolute weight during the period of acute inanition. In an earlier paper, Donaldson ('08) in table 4 gives the average weight of the spinal cord (at body weight of 245 grams) as 0.630 gram in 2 males, and 0.640 gram in 4 females. On the whole, therefore, we may conclude that the spinal cord during acute inanition undergoes little or no change in weight. Individual variations make comparisons with controls more or less uncertain, especially in a relatively small series of observations.

A somewhat different result is found in the chronic inanition series. Donaldson's ('09) data would indicate that the spinal cord in body weights corresponding to the average initial weight (214 grams) of this series would form an average weight of 0.593 gram or 0.28 per cent of the body weight. In an earlier paper, Donaldson ('08) in table 4 gives the weight of the spinal cord (at body weight of 215 grams) as averaging 0.590 gram in 5 males, and 0.630 gram in 3 females (corresponding theoretical weight by formula being 0.593 gram, sexes combined). The actual weight of the cord found at the end of the inanition period averages 0.569 gram, or about 0.43 per cent of the body weight. This would indicate a loss of about 4 per cent in the absolute weight of the spinal cord during chronic inanition. Thus in the spinal cord, as in the brain, there appears to be in the adult rat a tendency to greater loss in chronic than in acute inanition. In the young rat, however, this tendency is more than counterbalanced by the growth impulse, so that the spinal cord may gain in weight while the body weight is held constant (Donaldson '11; Jackson '15).

In any event, however, it is evident that the adult spinal cord, like the brain, shows but very slight if any loss in absolute weight during inanition, thus increasing markedly in relative (percentage) weight. This is in general agreement with the observations of Chossat ('43), Falck ('54), Lasarew ('97), Sedlmair ('99), Voit ('66), and Bechterew ('95) previously mentioned in the discussion of the brain.

EYEBALLS

The eyeballs in the acute inanition series (table 3) form an average of about 0.19 per cent of the body weight. In normal rats corresponding to their initial body weight, the average should be about 0.12 per cent (Jackson '13). Since the body weight has decreased about one-third (average 33.9 per cent) it is evident that the eyeballs must have remained nearly stationary in absolute weight, thus increasing their relative (percentage) weight by about one-half. In terms of absolute weight, there would appear to be a reduction from 0.298 gram (the theoretical weight corresponding to the initial body weight of 244 grams, according to Hatai '13) to 0.285 gram, the final average weight of the eyeballs (table 3). This would indicate a loss of 4.4 per cent.

A similar condition is found in the chronic inanition series. The eyeballs here form an average of about 0.20 per cent of the body weight, while the normal for the initial body weight averages about 0.13 per cent. (The higher figures in the chronic inanition series are due to the smaller initial body weight in this series.) Thus it is evident that, with a loss of about one-third (average 36.1 per cent) in the body weight during inanition, the absolute weight of the eyeballs has changed but little. In terms of absolute weight there would appear to be a reduction from about 0.280 gram (the theoretical weight corresponding to the average initial body weight of 214 grams, according to Hatai '13) to 0.2638 gram, the final average weight of the eyeballs (table 3). This would correspond to a loss of 5.8 per cent in the weight of the eyeballs during chronic inanition.

That the eyeballs lose little or nothing in absolute weight during inanition, increasing proportionately in relative (percentage) weight, is also indicated by the data of Falck ('54) for the dog, and Sedlmair ('99) for the cat. Bitsch ('95) finds the (absolute) weight of the eyeballs in the dog usually *increased* during inanition, which he suggested may be due to oedema.

THYROID GLAND

The thyroid gland in the acute inanition series (table 3) forms an average of 0.023 per cent of the body weight. The normal for the initial body weights would average about 0.015 per cent (Jackson '13). Since the body weight has been reduced one-third during inanition, this would indicate that there has been no loss in the absolute weight of the thyroid gland. In terms of absolute weight, the thyroid gland after acute inanition averages 0.0380 gram, which is almost identical with the theoretical weight corresponding to the average initial body weight of 244 grams (Hatai '13).

The results in chronic inanition are somewhat different. Here the thyroid gland forms an average of about 0.020 per cent of the body weight, the normal for the corresponding initial body weights being about 0.016 per cent. In this case the thyroid gland has apparently lost weight during chronic inanition, though relatively less than the body as a whole. According to Hatai ('13) the weight of the thyroid gland corresponding to the average initial body weight of 214 grams would be about 0.034 gram. The final absolute weight averages 0.0266 gram, indicating a loss of about 21.8 per cent in chronic inanition.

On account of the variability of the thyroid gland, however, and the difficulty in dissecting it out accurately, no final conclusions can be drawn from the limited number of observations at hand. As in the case of the brain, spinal cord and eyeballs, however, there appears to be a greater tendency to loss in weight of the thyroid gland during chronic inanition.

Excepting the observations by Falck ('54), indicating no change in the relative (percentage) weight of the thyroid gland during inanition in the dog, no data on this subject have been found in the literature. Traina ('04) cites data, however, upon the histological changes.

THYMUS

It is already known that the thymus in the albino rat normally undergoes a diminution in weight (due to age involution) after the age of 10 weeks (Jackson '13) or more precisely 85 days (Hatai '14). At the age of one year, it forms normally about 0.020 per cent of the body (Jackson). The thymus in the present acute inanition series forms an average of about 0.020 per cent of the body weight, while in the chronic inanition series the average is 0.021 per cent (table 3). The age of the rats used in the inanition experiments is unknown, but from the body weight it is probable that few of them were less than a year old. Therefore while hunger is known to produce a marked involution of the thymus (Hammar), it is probable that in the present case the involution had already been produced by age, and was not caused by inanition. According to Jonson ('09), in young rabbits the weight curves of fat and thymus are similar in *chronic* inanition, but the fat decreases somewhat more rapidly in *acute* inanition.

HEART

The heart in the acute inanition series forms an average of about 0.43 per cent of the body weight, and about 0.42 per cent in the chronic inanition series (table 3). The normal for corresponding initial body weights would average about 0.43 per cent (Jackson '13) or about 0.38 per cent, according to Hatai's ('13) data. Thus it is evident that the heart during inanition has lost weight nearly in the same proportion as the whole body.

Using Hatai's ('13) curves to determine the normal at corresponding initial body weights, the absolute weight of the heart apparently decreases from about 0.925 to 0.6687 gram (loss of 27.7 per cent) in the acute inanition series; and from about 0.830 to 0.5577 gram (loss of 32.8 per cent) in the chronic inanition series. As in the case of the viscera previously considered, the loss is apparently slightly greater relatively in chronic inanition.

The statements in the literature generally indicate that the heart during inanition loses somewhat less in weight than the body as a whole, and increases accordingly in relative (percentage) weight. This would appear to be the case in man (Aschoff '11; Lustig '02), cat (Voit '66; Sedlmair '99), newborn kitten (v. Bechterew '95) dog (Falck '54), guinea-pig (Lasarew '97), rabbit (Bowin '80), and in thin compared with fat steers (data from Missouri Agricultural Experiment Station). The data of Chossat ('43) for pigeons, however, would indicate a reduction in heart weight relatively slightly greater than in the body as a whole.

LUNGS

The lungs of the rat are quite variable in weight, owing to the frequency of infection, which may affect their weight even when the lesions are slight. The average for the lungs in the acute inanition series is 0.61 per cent of the body weight, and 0.55 per cent in the chronic inanition series (table 3). Since the normal lungs corresponding to the average initial body weight form an average of about 0.60 per cent (Jackson '13), it appears (although the evidence is insufficient for final conclusions) that during inanition, both acute and chronic, in adult albino rats the lungs lose in weight in about the same proportion as the whole body, their relative (percentage) weight remaining nearly the same. Taking the normal weight of the lungs at corresponding body weights from Hatai's ('13) curve, there is apparently a decrease from about 1.40 to 0.968 gram (loss of 30.9 per cent) in the acute inanition series; and from about 1.24 to 0.743 gram (loss of 40 per cent) in the chronic series. Again, as in the case of the viscera previously considered, the loss appears relatively greater during chronic inanition.

Comparatively few data are found in the literature concerning the weight of the lungs in inanition. In pigeons, Chossat ('43) found a loss of 22.4 per cent in the lungs, compared with 40 per cent in the entire body weight. Thus their relative (percentage) weight is considerably increased, which was also observed by Voit ('66) and Sedlmair ('99) in the cat, and von Bechterew ('95) in newborn kitten. In the dog, however, Falck's ('54) data indicate a loss relatively slightly greater than that of the body, with a corresponding decrease in percentage weight.

LIVER

The liver in the acute inanition series (table 3) forms an average of about 2.88 per cent of the body weight. Since the normal for the corresponding initial body weights is about 4.5 per cent in the acute inanition series, and 4.3 per cent in the chronic (Jackson '13) it is evident that the liver has apparently lost in weight relatively more than the body as a whole. In the chronic inanition series, however, the weight of the liver is more variable, and the average (3.98 per cent) is but little below the normal. As the liver is normally subject to great individual variations in weight (Jackson '13), however, caution should be observed in drawing final conclusions.

In terms of absolute weight, using my (Jackson '13) data for comparison, it appears that the liver decreases from about 10.98 to 4.587 grams (loss of 58 per cent) in the acute inanition series, and from 9.20 to 5.219 grams (loss of 43 per cent) in the chronic inanition series. It may also be remembered that in the series investigated by Hatai ('13), the average weight of the normal liver was found distinctly higher than in my series; and the loss in weight, estimated upon this basis, would be considerably greater.

With the exception of the adipose tissue, the thymus (in young animals) and occasionally the spleen, all investigators agree that the loss of weight in the liver is relatively greater than that in any other organ. Thus the liver decreases in relative (percentage) weight, as has been observed in man (Aschoff '11), white mouse (Cesa-Bianchi '09), pigeon (Chossat '43), rabbit (Pfeiffer '87), cat (Voit '66; Sedlmair '99), dog (Falck '54), newborn cat and dog (v. Bechterew '95), guinea-pig (Lasarew '97) and in thin steers, compared with fat (data from Missouri Agricultural Experiment Station). While the loss is practically always relatively greater than in the body as a whole, the amount of loss is quite variable.

Since the loss in weight of the liver (unlike all viscera previously considered) is relatively greater in acute inanition it seems probable that the greatest loss occurs during the earliest stages of inanition, when the liver yields its store of easily available food material (glycogen, fat, etc.). Thus Lasarew ('97) found that during the early period of inanition in the guinea-pig, while the body weight lost 10 per cent, the liver lost 18 per cent of its weight, or relatively nearly twice as much. Toward the end of the inanition period, on the contrary, the liver apparently lost relatively only half as much as the whole body.

SPLEEN

In the acute inanition series (table 3), the spleen forms an average of 0.21 per cent of the body. This is below the normal for the average corresponding initial body weight, in which case the average is 0.27 per cent. In the chronic inanition series, however, the average (0.30 per cent) is slightly higher than the normal (Jackson '13). The individuals in both acute and chronic inanition, however, are exceedingly variable, as seen in the table. This is also true of the normal spleen, which is one of the most variable organs in the body (Jackson '13). The number of observations is therefore insufficient for final conclusions concerning the effect of inanition upon the weight of the spleen in the albino rat.

In terms of absolute weight, taking Hatai's ('13) curve for the normal, there is apparently a decrease in the weight of the spleen from about 0.645 to 0.3177 gram (loss of 51 per cent) in the acute inanition series; and from 0.570 to 0.4056 gram (loss of 29 per cent) in the chronic series.

That the spleen loses heavily during inanition, losing in relative as well as absolute weight, has been found in man (Aschoff '11; Stschastny '98), pigeon (Chossat '43), rabbit (Bowin '80), and cat (Voit '66; Sedlmair '99); while a decrease less marked than in the whole body (relative increase) appears in the dog (Falck '54) and in thin steers, compared with fat (data from Missouri Agricultural Experiment Station). Data from von Bechterew ('95) indicate a relative increase in the spleen of newborn kittens during inanition, but a decrease in puppies. These apparently conflicting statements are perhaps to be explained largely by the great variability of the spleen, making comparison with controls uncertain. In addition, however, there is the possibility that the loss in the spleen may vary according to the character and stage of inanition. Thus Lasarew ('97) in the guinea-pig found the greatest loss in weight of the spleen to occur in the middle period of inanition (second period of 10 per cent loss in body weight), during which the spleen lost 31 per cent in weight.

STOMACH AND INTESTINES

The stomach and intestines (including mesentery and pancreas) together with their content (table 3) form an average of about 5.2 per cent of the body weight in the acute inanition series, and 6.3 per cent in the chronic inanition series. Since the average normal for the initial body weights is about 9.0 per cent (Jackson '13), it is evident, as might be expected, that the stomach and intestines with contents have lost weight in relatively much greater proportion than has the body as a whole. The loss affects not only the contents, but the empty tract, which has apparently decreased from about 6.0 per cent (normal at the initial body weight) of the body weight to 3.25 per cent (acute inanition series) or 3.5 per cent (chronic series). There is apparently not much difference in this respect between the chronic and acute series. A relatively small part of the loss is in the mesenteric fat.

In terms of absolute weight, taking Hatai's ('13) curve for the normal, there is apparently a decrease in the weight of the empty alimentary canal from about 11.65 to 5.01 grams in the acute inanition series, and from 10.70 to 4.54 grams in the chronic series. This would correspond to a loss of about 57 per cent in each series.

Data in the literature are very scarce concerning changes in weight of the alimentary canal during inanition. According to Falck ('54), the loss appears to be nearly proportional to that of the entire body (relative weight increasing from 8.4 to 8.7 per cent). This appears to be true also for the cat according to Sedlmair ('99), although a much smaller loss was found by Voit ('66). Unpublished data on a series of steers (Missouri Agricultural Experiment Station) indicate that the relative weight of the (empty) intestines (without fat) is variable, but tends to be greater in thin than in fat animals.

SUPRARENAL GLANDS

The suprarenal glands must be considered separately in the sexes on account of a difference in relative weight (Jackson '13). In normal rats corresponding to the initial body weights of the present series, the average for the suprarenal glands of the male is about 0.17 per cent of the body weight, and for the female about 0.26 per cent. The four females of the acute inanition series (none present in the chronic series) show an average of 0.30 per cent of the body weight (table 3), but the number is too small for definite conclusions. The males show an increase (from 0.17 per cent) to an average of 0.25 per cent in both acute and chronic inanition series (table 3). It would therefore appear that the loss in absolute weight has been less than in the body as a whole in the female with little or no loss in the male.

In terms of absolute weight, taking Hatai's ('13) curve for the normal, it appears that in the male rat the suprarenals have *increased* from about 0.0390 to 0.0396 grams (gain of 1.5 per cent) in the acute inanition series; and *decreased* from about 0.0360 to 0.0328 grams (loss of 8.9 per cent) in the chronic series. The apparent increase during acute inanition is probably not significant. A larger number of observations is necessary to determine the matter.

No data concerning the weight of the suprarenal glands during inanition have been found in the literature, although Traina ('04) cites several investigations on the histological changes.

KIDNEYS

The kidneys (table 3) in the acute inanition series form an average of 0.97 per cent of the body weight and in the chronic series 1.00 per cent. According to Jackson ('13) the normal for the initial body weights would average about 0.95 per cent (although Hatai's data would place it at about 0.84 per cent). It therefore appears that the kidneys have lost weight to nearly the same extent relatively as the body in general, thus gaining but slightly in relative (percentage) weight.

If Hatai's ('13) curve be taken as the normal, in absolute weight the kidneys would apparently decrease from about 2.04 to 1.520 grams (a loss of 25.5 per cent) during acute inanition; and from 1.80 to 1.317 grams (a loss of 26.8 per cent) in the chronic inanition series. If my data indicating a higher normal were taken, the loss would be correspondingly greater.

Considering the importance of the kidneys, there is in the literature a surprising lack of data concerning their weight during inanition. That they lose in weight relatively somewhat less than the body as a whole (thus gaining in percentage weight) is indicated in the pigeon (Chossat '43), cat (Voit '66) and dog (Falck '54). There appears also a slight relative increase in the kidney weight of thin steers compared with fat (data from Missouri Agricultural Experiment Station). On the other hand, Sedlmair's ('99) data for starved cats indicate no change in relative (percentage) weight in one case and a slight decrease in another.

GONADS

a. Female. The two observations recorded for the weight of the ovaries, 0.051 per cent and 0.033 per cent of the body weight are both above the normal average (0.025 per cent), but are of course entirely too few to be significant.

b. Male. At the age of one year (body weight, 210 grams) the normal testes and epididymi form an average of about 1.20 per cent of the body weight, of which approximately one-fourth (0.30 per cent) belongs to the epididymi, and three-fourths (0.90 per cent) to the testis (Jackson '13). Hatai's ('13) data would put the testis a little higher (about 1.05 per cent). In the acute inanition series (table 3) the average relative weight for the testes is 1.12 per cent, and for the epididymi is 0.39 per cent of the body weight. In the chronic series, the average for the testes is 1.02 per cent, and for the epididymi 0.33 per cent. It would therefore appear that during inanition the loss in weight of both testes and epididymi is relatively not very different from that in the body as a whole, and is (like that of the majority of the viscera) more marked in chronic than in acute inanition. On account of the variability of these organs, however, more data are needed before final conclusions can be justified.

In terms of absolute weight, taking Hatai's ('13) data for the normal at the initial body weight, the testis would appear to decrease from 2.50 to 1.756 grams (a loss of 29.8 per cent) in the acute inanition series; and from 2.27 to 1.355 grams (a loss of 40.3 per cent) in the chronic inanition series.

Although numerous investigations have been made upon the histological changes in the gonads during inanition (Traina '04), the only observations found concerning their weight are that by Falck ('54) showing the relative (percentage) weight of the testis in the dog to remain apparently unchanged, and that by (Voit '66) showing a relative decrease in the testis of the cat.

HYPOPHYSIS

In the case of the hypophysis, as with the suprarenal glands, there is normally a sexual difference to be considered (Hatai '13). When the data in table 3 are compared with Hatai's chart 10, it will be found that the absolute weights of the hypophysis, in both acute and chronic inanition, correspond fairly well with those of the normal gland at the *final* body weight. That is, the weight of the hypophysis during inanition has apparently decreased in nearly the same proportion as the whole body, so the relative weight is but little changed. As calculated from Hatai's data, the (male) hypophysis would form about 0.0036 per cent of the body at the average initial body weight, as compared with 0.0043 per cent found in the acute inanition series, and 0.0045 per cent in the chronic series.

In terms of absolute weight, the (male) hypophysis has apparently decreased from about 0.0093 to 0.0069 gram, the average of the acute inanition series (loss of 26.1 per cent); and from 0.0079 to 0.0059 gram (loss of 25.3 per cent) in the chronic inanition series. A larger number of observations would of course be necessary to determine the result with precision.

No data have been found in the literature concerning the weight of the hypophysis during inanition.

DISCUSSION

The changes in the average relative weights of the various organs and systems as a result of inanition in the adult albino rat are summarized in table 2.

While no great emphasis can be laid upon the exactness of the figures shown in table 2, it is evident that with respect to relative loss in weight during inanition, the organs may be divided into three groups. In the first group, which includes the suprarenals, thyroid, skeleton, eyeballs, spinal cord and brain, and thymus, there is but little (if any) loss in absolute weight during inanition, and a corresponding increase in relative (percentage) weight. In general, there is a relatively greater loss during chronic

TABLE 2

ORGAN OR SYSTEM	NORMAL PERCENTAGE OF BODY AT INITIAL BODY WEIGHT	PERCENTAGE OF BODY AFTER INANITION		PERCENTAGE LOSS OF ORGAN DURING INANITION	
		Acute	Chronic	Acute	Chronic
Suprarenals (male).........	0.0170	0.0220	0.0260	+1.5(?)	−8.9
Thymus.........	0.0200	0.0200	0.0210	0(?)	0(?)
Thyroid gland...	0.0150(0.016)	0.0230	0.0200	0(?)	−21.8
Spinal cord......	0.2500(0.290)	0.4000	0.4300	0(?)	−4.0
Ligamentous skeleton.......	10.0000	15.0000	16.4000	−0.4	+1.8(?)
Eyeballs.........	0.1200(0.130)	0.1900	0.2000	−4.4	−5.8
Brain............	0.7800(0.870)	1.1700	1.3300	−5.1	−6.6
Kidneys.........	0.9500	0.9600	1.0000	−25.5	−26.8
Hypophysis......	0.0036	0.0043	0.0045	−26.1	−25.3
Heart...........	0.4300	0.4400	0.4200	−27.7	−32.8
Testes...........	0.9000	1.0600	1.0100	−29.8	−40.3
Lungs...........	0.6000	0.6100	0.5500	−30.9	−40.0
Musculature.....	45.0000	47.5000	43.0000	−30.9	−40.8
Integument......	18.0000	19.1000	17.8000	−31.2	−38.5
Whole body......	*100.0000*	*100.0000*	*100.0000*	*−33.9*	*−36.1*
Spleen...........	0.2700	0.2100	0.3100	−51.0	−29.0
Stomach—intestines..........	6.0000	3.4000	3.5000	−57.0	−57.0
Liver............	4.5000	3.1000	4.0000	−58.0	−43.0

inanition, which is especially marked in the case of the thyroid gland. The thymus, having already undergone age involution, is affected but slightly, if at all.

In the second group, which includes the kidneys, hypophysis, heart, testes, lungs, musculature and integument, the loss in absolute weight during inanition is more nearly in proportion to that of the whole body, so their relative (percentage) weight is usually not greatly changed. In all except the hypophysis, however, the loss is relatively greater during chronic inanition. Especially the lungs, testes, integument and musculature appear to lose markedly during chronic inanition.

In the third group, including the spleen, liver and alimentary canal, the loss in absolute weight is relatively much greater than in the body as a whole, so they decrease in relative as well as in absolute weight. The liver and spleen are exceptional, however, in that their loss is apparently relatively greater in acute than in chronic inanition. In fact, in chronic inanition the spleen apparently belongs with the second group.

The variability of the organs as to loss of weight during inanition has been explained in two ways: Manassein ('69) noted that those organs which are most active in the organism lose least during inanition. A more rational explanation is that of Paschutin ('81), according to whom the various organs lose in proportion to their storage content of available food supply. The various proteids, fats and carbohydrates are dissolved and carried away by the circulation at different times and with different degrees of rapidity. Thus the variability in the loss of weight in different organs and in different types of inanition would be ultimately explained primarily upon a chemical basis.

SUMMARY

The principal results of the present paper may be briefly summarized as follows:

1. During both acute and chronic inanition there is apparently a slight increase in the ratio of tail length to body length. This is probably due to a decrease in the trunk length during inanition.

2. The head and fore limbs during inanition lose relatively less than the body as a whole, and therefore increase in relative (percentage) weight. The hind limbs nearly maintain their original relative weight (slight increase during acute inanition), while the trunk decreases in relative weight.

3. Of the systems—integument, skeleton, musculature, viscera and 'remainder'—the integument and musculature lose relatively in nearly the same proportion as the whole body, slightly less during acute inanition and slightly more during chronic inanition. The skeleton nearly maintains its original absolute weight, and therefore increases markedly in relative (percentage) weight. There is a marked decrease in the 'remainder,' probably due chiefly to loss of fat. The visceral group as a whole undergoes little change in relative weight, showing a slight decrease during acute inanition. This decrease is due to the large size of the liver, which undergoes a greater loss in acute than in chronic inanition. The majority of the viscera, on the other hand, show a greater loss during chronic inanition.

4. As to relative loss of weight during inanition, the individual viscera may be divided into three groups: (1) the suprarenal glands, thyroid glands, eyeballs, spinal cord and brain lose but very little (if any) in absolute weight, and therefore increase correspondingly in relative (percentage) weight. The thymus has already undergone age involution, and is therefore unaffected. (2) The kidneys, heart, lungs, hypophysis and testes lose more nearly in proportion to the entire body (in general, somewhat more during chronic inanition), and therefore do not change greatly in relative (percentage) weight. (3) The spleen (in acute inanition), liver and alimentary canal (both empty and with contents) lose relatively much more heavily than the whole body, and therefore decrease in relative (percentage) as well as in absolute weight.

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TABLE 3

Acute and chronic inanition

RAT NO. AND SEX	LENGTH OF BODY	LENGTH OF TAIL	RATIO OF TAIL TO BODY	INANITION PERIOD	ORIGINAL GROSS BODY WEIGHT	FINAL GROSS (AND NET) BODY WEIGHT	LOSS OF BODY WEIGHT	HEAD		FORE-LIMBS	
								Weight	Percent of body	Weight	Percent of body
	cm.	*cm.*		*days*	*grams*	*grams*	*per cent*	*grams*		*grams*	
S 18 f.	17.5	17.5	1.00	Acute, 8.0	196.0	121.0(117.2)	38.0	16.2	13.9		
M 8 m.	17.1	17.3	1.01	10.0	221.0	132.0(128.9)	40.0	14.1	10.9	9.2	7.1
O 6 f.	18.0	15.8	0.88	12.0	193.0	132.0(129.5)	32.0	17.4	13.4		
M 7 m.	16.6	16.0	0.96	9.0	216.0	134.0(131.2)	38.0	14.2	10.8	8.5	6.5
M 9 m.	15.3	16.5	1.08	6.0	190.0	137.0(134.0)	28.0	14.8	11.1	8.7	6.5
O 5 f.	20.0	18.0	0.90	8.0	182.0	136.0(135.0)	25.0	16.4	12.1		
M 10 m.	18.1	17.2	0.95	9.0	221.0	142.0(139.4)	36.0	14.8	10.7	10.3	7.4
M 1 m.	20.5	19.5	0.95	9.0	248.0	168.0(165.2)	32.0	17.0	10.3	13.8	8.4
M 2 m.				11.0	254.0	170.0(167.2)	33.0	18.9	11.3	12.7	7.6
S 26 m.	20.5	18.0	0.88	8.0	284.0	174.0(171.5)	39.0	18.7	10.9		
S 16 f.	19.5	17.5	0.90	9.0	267.0	190.0(186.0)	29.0	20.3	10.9		
S 25 m.	20.5	19.0	0.93	9.0	328.0	202.0(198.3)	35.0	22.1	11.1		
S 27 m.	21.5	18.0	0.84	8.0	320.0	223.0(219.0)	30.0	23.6	11.0		
M 13 m.	21.3	19.6	0.92	6.0	333.0	240.0(237.0)	28.0	23.7	10.0	16.8	7.1
S 28 m.	23.0	19.5	0.85	10.0	367.0	243.0(240.0)	34.0	24.6	10.3		
Average (acute)	19.2	17.8	0.93	Acute, 9.0	255.0 (244 for viscera)	170.0(166.0) 160.0(157.4) for viscera	33.1 (33.9 for viscera)	18.47	11.2 (10.7 for limbs and trunk	11.4	7.2
M 3 m.	17.5	16.5	0.94	Chronic, 36.0	188.0	125.0(122.1)	33.5	13.9	11.4	9.8	8.0
M 12 m.	17.3	17.1	0.99	33.0	200.0	128.0(124.6)	36.0	14.2	11.4	7.8	6.2
M 5 m.	19.0	18.5	0.97	36.0	205.0	129.0(126.6)	37.0	15.2	12.0	10.4	8.2
M 4 m.	17.5	17.0	0.97	41.0	205.0	134.0(129.2)	35.1	13.5	10.5	7.4	5.7
M 6 m.	17.5	16.7	0.95	35.0	220.0	138.0(134.1)	37.2	15.3	11.4	9.5	7.1
M 11 m.	19.0	18.6	0.98	33.0	264.0	163.0(158.5)	37.5	18.3	11.5	9.8	6.2
Average (chronic)	18.0	17.4	0.97	Chronic, 35.7	213.7	136.2(132.5)	36.1	15.1	11.4	9.1	6.9

TABLE 3—*Continued*

RAT NO. AND SEX	HIND-LIMBS		TRUNK		INTEGUMENT		LIGAMENTOUS SKELETON		CARTILAGINOUS SKELETON		DRY SKELETON	
	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body
	grams		*grams*		*grams*		*grams*		*grams*		*grams*	
S 18 f.					23.06	19.7	20.30	17.4				
M 8 m.	22.5	17.4	83.1	64.5	25.50	19.8	21.60	16.7	16.15	12.5	7.321	5.68
O 6 f.					24.20	18.6	19.50	15.1				
M 7 m.	23.9	18.2	84.6	64.5	24.80	18.9	19.70	15.4	15.19	11.6	7.224	5.51
M 9 m.	23.8	17.8	86.7	64.7	25.20	18.8	19.50	16.4	14.81	11.1	7.094	5.30
O 5 f.					22.00	16.3	15.20	11.3				
M 10 m.	24.3	17.5	90.0	64.7	27.30	19.6	21.70	15.6	15.05	10.8	7.325	5.27
M 1 m.	28.6	17.3	105.8	64.1	32.20	19.5	26.00	15.8	19.30	11.7	10.516	6.37
M 2 m.	27.6	16.5	108.0	64.6	35.00	21.0	21.00	12.6	14.00	8.4	7.741	4.64
S 26 m.					33.36	19.5	29.34	17.1				
S 16 f.					32.48	17.5	28.13	15.1				
S 25 m.					39.78	20.2	25.48	12.9				
S 27 m.					40.63	18.5	33.03	15.1				
M 13 m.	42.6	18.0	153.9	64.9	46.70	19.8	37.60	15.9	24.37	10.3	13.324	5.62
S 28 m.					41.43	17.3	40.01	16.7				
Average (acute)	27.6	17.5	101.7	64.6 (64.1)	31.60	18.9 (19.1 for viscera)	25.40	15.3 (15.0 for viscera)	16.98	10.9	8.748	5.48
M 3 m.	18.5	15.2	79.9	65.5	19.50	16.0	18.50	15.2	14.00	11.5	6.511	5.34
M 12 m.	20.7	16.6	81.9	65.5	21.40	17.1	21.40	17.1	14.60	11.7	7.156	5.72
M 5 m.	19.0	15.0	82.0	64.6	24.50	19.3	22.50	17.8	16.65	13.2	9.310	7.33
M 4 m.	15.9	12.3	92.4	71.4	18.90	14.7	18.20	14.1	16.10	12.5	7.318	5.67
M 6 m.	21.1	15.7	88.2	65.8	25.50	19.1	21.90	16.3	16.20	12.1	7.690	5.74
M 11 m.	26.5	16.7	103.9	65.3	32.70	20.6	28.30	17.8	21.52	13.5	11.015	6.38
Average (chronic)	20.3	15.3	88.05	66.4	23.70	17.8	21.80	16.4	16.5	12.4	8.167	6.03

TABLE 3—*Continued.*

RAT NO. AND SEX	STOMACH-INTESTINES (FILLED)		STOMACH-INTESTINES (EMPTY)		SUPRARENALS		KIDNEYS		TESTIS		EPIDIDYMI		HYPOPHYSIS	
	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body
	grams		*grams*		*grams*		*grams*		*grams*		*grams*		*grams*	
S 18 f.	8.360	7.15	4.530	3.88	0.0468	0.040	1.075	0.92	(0.0600)*	(0.051)*				
M 8 m.	6.655	5.16	3.585	2.78	0.0380	0.029	1.374	1.07	1.754	1.36	0.4666	0.36	0.0066	0.0051
O 6 f.	8.270	6.38	4.680	3.60	0.0330	0.025	1.374	1.06						
M 7 m.	6.509	4.96	3.729	2.84	0.0414	0.032	1.235	0.94	1.675	1.28	0.4860	0.37	0.0060	0.0046
M 9 m.														
O 5 f.	8.720	6.46	7.250	5.37	0.0420	0.031	1.484	1.10						
M 10 m.	6.860	4.94	4.290	3.08	0.0344	0.025	1.410	1.01	2.179	1.57	0.5590	0.43	0.0068	0.0049
M 1 m.	6.090	3.69	3.280	1.99	0.0414	0.025	1.513	0.92	1.502	0.91	0.4490	0.27	0.0070	0.0043
M 2 m.	7.040	4.22	4.290	2.57	0.0338	0.020	1.505	0.90	1.124	0.90	0.4680	0.67	0.0066	0.0040
S 26 m.	7.100	4.15	4.550	2.66	0.0398	0.023	1.557	0.91	1.905	1.11	0.7640	0.45	0.0076	0.0045
S 16 f.	11.980	6.44	8.610	4.63	0.0416	0.022	1.634	0.88	(0.0616)*	(0.033)*			0.0146	0.0078
S 25 m.	8.540	4.31	5.070	2.56	0.0458	0.023	1.929	0.97	1.479	0.75	0.4120	0.21	0.0048	0.0024
S 27 m.	10.320	4.71	6.270	2.86	0.0424	0.019	2.150	0.98	2.430	1.11	0.7210	0.33	0.0096	0.0044
M 13 m.														
S 28 m.														
Average (acute)	8.040	5.21	5.010	3.25	m0.0396 f0.0409	0.025 0.030	1.520	0.97	1.756	1.12	0.5407	0.39	m0.0069 f0.0146	0.0043 0.0078
M 3 m.	10.480	8.60	6.530	5.40	0.0252	0.021	1.252	1.04	0.682	0.56	0.3060	0.25	0.0058	0.0048
M 12 m.	7.060	5.70	3.660	2.90	0.0306	0.025	1.418	1.13	1.829	1.46	0.4400	0.35	0.0052	0.0042
M 5 m.	6.030	4.80	3.660	2.90	0.0320	0.025	1.153	0.91	1.201	0.95	0.3910	0.31	0.0058	0.0046
M 4 m.	8.950	6.90	4.220	3.30	0.0496	0.038	1.465	1.13	1.113	0.86	0.3430	0.27	0.0062	0.0048
M 6 m.	8.480	6.30	4.580	3.40	0.0270	0.020	1.235	0.92	1.708	1.27	0.6160	0.46	0.0060	0.0045
M 11 m.	9.080	5.70	4.580	2.90	0.0326	0.021	1.379	0.87	1.597	1.00	0.5410	0.34	0.0064	0.0040
Average (chronic)	8.35	6.30	4.540	3.50	0.0328	0.025	1.317	1.00	1.355	1.02	0.4400	0.33	0.0059	0.0045

*Ovaries.

Reprinted from THE JOURNAL OF EXPERIMENTAL ZOÖLOGY, Vol. 19, No. 2,
August, 1915

CHANGES IN THE RELATIVE WEIGHTS OF THE VARIOUS PARTS, SYSTEMS AND ORGANS OF YOUNG ALBINO RATS HELD AT CONSTANT BODY-WEIGHT BY UNDERFEEDING FOR VARIOUS PERIODS

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When young, actively growing animals are held at constant body-weight by underfeeding for considerable periods, one of three results might be expected *a priori* concerning the weights of the various organs and parts. (1) Since the body-weight remains constant, the weights of the individual organs and parts might also remain constant. (2) Since the normal growth rates are known to vary in the different organs and parts, one might expect those organs with the strongest normal growth tendency to increase in weight at the expense of the remainder of the body. (3) Since, however, the food-supply available is insufficient for both maintenance and growth, the animals are actually in a condition of chronic inanition; and changes might be expected to occur corresponding to those which have been observed in adults during inanition.

A few observations have been recorded upon changes in young animals in certain organs under these conditions, notably by Waters ('08), Aron ('11 and '14) and Donaldson ('11). A more extended and complete analysis of the changes in the organism under these conditions seemed highly desirable, and therefore the present investigation was undertaken. The work was begun at the University of Missouri, and continued at the University of Minnesota with the aid of a special grant from the research fund of the Graduate School. This grant was used to employ a research assistant, who cared for the animals and assisted in the dissections, weighings, calculations, etc. An abstract of the present paper has been published (Jackson '15 b).

MATERIAL AND METHODS

The albino rat (Mus norvegicus albinus) was chosen for use in this experiment. It is a convenient animal on account of its comparatively small size, rapid growth and hardiness under experimental conditions. It is also practically the only mammal whose growth norm (including variability) for the whole body and for the various parts, systems and organs throughout the post-natal life cycle is even approximately known. The effects of inanition upon the adult rat have also been worked out (Jackson '15 a, '15 c) and are valuable for comparison.

The material used in the present experiment included ten litters (and one individual from an eleventh litter) as shown in table 1.

TABLE 1

Litters used in experiments

LITTER NO.	SOURCE	NO. AND SEX OF EACH LITTER	NO. TEST ANIMALS	CONSTANT WEIGHT PERIOD	NO. CONTROL ANIMALS	KILLED AT AGE OF
L 4...	Missouri	1 M. 4 F.	1 M. 3 F	3–6 wks.	1 F.	6 wks.
L 2...		3 M. 2 F.	1 M. 1 F.	6–32 wks.	2 M. 1 F.	32 wks.
L 3...		4 M. 2 F.	2 M. 1 F.	10–35 wks.	1 M. 2 F.	35 wks.
S 5...	Minneapolis	3 M. 8 F.	1 M. 1 F.	3–8 wks.	1 M. 1 F.	3 wks.
			5 F.	3–10 wks.	1 M. 1 F.	10 wks.
S 6...		2 M. 4 F.	1 M. 3 F.	3–10 wks.	1 M. 1 F.	10 wks.
S 7...		4 M. 2 F.	3 M. 1 F.	3–10 wks.	1 M. 1 F.	3 wks.
S 11..		2 M. 4 F.	1 M. 2 F.	3–10 wks.	1 M. 1 F.	3 wks.
					1 F.	10 wks.
S 12..		5 M. 3 F.	3 M. 3 F.	3–10 wks.	1 M.	3 wks.
					1 M.	10 wks.
S 10..		1 M. 2 F.	1 F.	3–16 wks.	1 M. 1 F.	3 wks.
28....		4 M. 4 F.	2 M. 2 F.	3-6 wks.	1 M. 1 F.	3 wks.
			1 F.	3–8 wks.	1 M.	6 wks.
22....		1 F.	1 F.	3–13 wks.		
Total		29 M. 36 F.	15 M. 25 F.		13 M. 12 F.	

As noted in this table, three of the litters are from the rat colony at the University of Missouri, and the remainder from a local colony at the University of Minnesota. There are included twenty-nine males and thirty-six females, a total of sixty-five. In addition, two rats not listed in tables 1 and 2 were used as additional controls at three weeks in the study of the skeleton (table 7).

In most cases, the experiment began when the rats were three weeks of age (time of weaning). These rats were held at con-

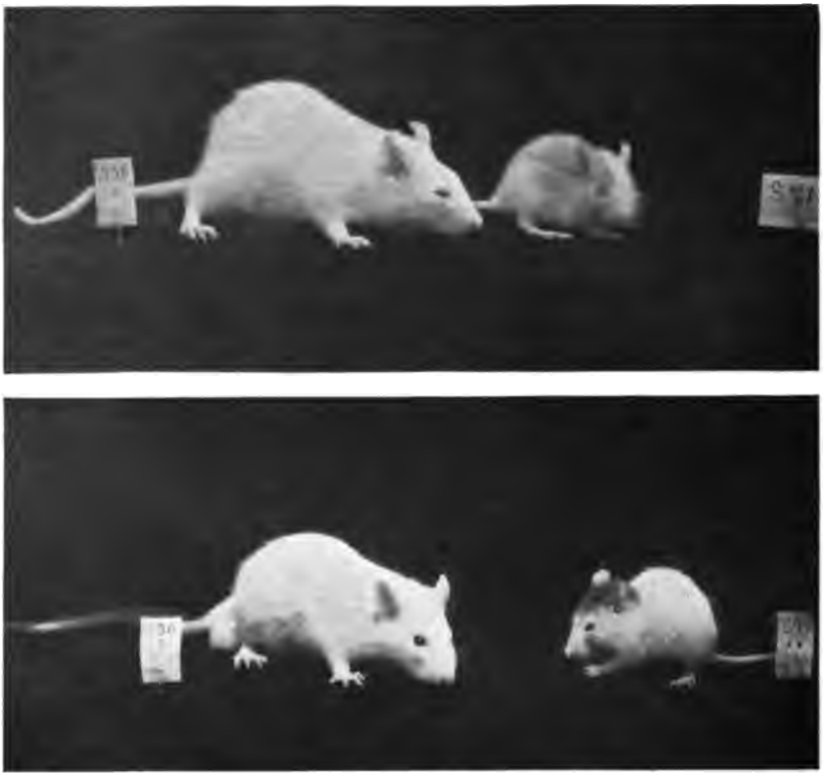

Fig. 1 Photograph showing four albino rats at the age of ten weeks. The larger rats represent the normal, full-fed controls. The smaller rats are from the same litter, but have been held by underfeeding at constant body-weight since the age of three weeks. The rats are pure albinos (Mus norvegicus albinus) the dark spot on the head of one being an artificial mark of identification.

stant body-weight for varying periods, from the age of three weeks up to the age of six weeks (8 rats) to eight weeks (3), to ten weeks (22), to thirteen weeks (1) and to sixteen weeks (1). A few were held at constant body-weight beginning at later periods,—from age of six weeks to age of thirty-two weeks (2), and from age of ten weeks to age of thirty-six weeks (3). Controls (25 in all) were also killed at the beginning and at the end of these time periods. At ten weeks of age, the normal controls are half-grown rats, sexually mature, of nearly adult proportions, and five or six times as heavy as those held at constant body-weight from the age of three weeks (fig. 1). In addition, a large number of observations upon the normal rat previously published (by Donaldson, Hatai, Jackson, Lowrey and others) are available for comparison. Of the animals used in this experiment, the distribution of sexes is given in table 1.

In table 2, the net body-weight (gross body-weight, including intestinal contents, is slightly higher), of the animals used is indicated. At each time period the average weight (and range) for each sex is given, for both controls and test animals held at constant body-weight. The cards containing the original individual records for all animals used will be deposited in The Wistar Institute of Anatomy, where they may be consulted if desired.

The rats were kept in ordinary wire cages (with wire-net bottoms, allowing the feces to drop through) and were individually weighed daily before feeding. Those under experiment

TABLE 2

Net body-weight of rats when killed; average weight and range indicated

CONTROLS (FULL-FED)			EXPERIMENTED (BODY-WEIGHT HELD CONSTANT)		
Controls killed at	No. and sex	Weight and range: grams	Weight and range: grams	No. and sex	Test animals constant for
3 weeks..	6 M.	24.4 (19.0-32.6)			
	5 F.	24.7 (21.1-30.4)			
6 weeks..	1 M.	42.3	22.4 (20.5-23.6)	3 M.	3-6 weeks
	1 F.	42.4	22.0 (20.6-23.8)	5 F.	
8 weeks			18.1	1 M.	3-8 weeks
			21.2 (17.8-24.6)	2 F.	
10 weeks	3 M.	155 (141-167)	24.7 (20.7-31.4)	8 M.	3-10 weeks
	3 F.	114 (109-118)	23.3 (18.5-31.0)	14 F.	
			25.5	1 F.	3-13 weeks
			26.0	1 F.	3-16 weeks
32 weeks.	2 M.	209 (203-215)	43.7	1 M.	6-32 weeks
	1 F.	166	50.4	1 F.	
35 weeks.	1 M.	238	79.6 (73.6-85.5)	2 M.	10-35 weeks
	2 F.	158 (153-162)	74.2	1 F.	
Total	13 M.			15 M.	
	12 F			25 F.	

were fed an amount just sufficient to hold them constant at the initial body-weight. Of course slight fluctuations in the gross weight were unavoidable, but they rarely exceeded one gram above or below the initial weight. The food used was in all cases whole wheat (Graham) bread soaked in whole milk. Previous experience has shown that, at least up to the age of one year, albino rats thrive and develop normally upon this simple diet. Water *ad libitum* was also supplied.

It is a curious fact that under these circumstances the amount of food necessary for maintenance of body-weight apparently decreases as the experiment proceeds. Thus rats of about 25 grams gross body-weight when three weeks of age at the beginning of the experiment will at ordinary room temperature require about 5 grams of milk-soaked bread daily for maintenance. Later, this will usually decrease to an average of about 3 grams toward the age of ten weeks.[1] This is the opposite of what might be expected: (1) because at later periods the amount of available food supply stored in the body has been greatly diminished; and (2) because the animals held at constant body-weight almost invariably become much more active, requiring a greater expenditure of energy. Possibly the smaller amount of food required to maintain the animals at the later periods may be due to a greater absorption of water, thus maintaining a body-weight which would otherwise decline with the given amount of food. It is well known that during inanition in general the percentage of water-content of the body increases, and it is quite probable that during chronic inanition resulting from maintenance of a young, growing animal at constant body-weight the amount of living protoplasm in the body decreases. If the amount of metabolism is thereby decreased, a smaller food-supply would suffice for maintenance.

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[1] Two examples may be cited. Six rats of litter No. 12 were held at constant body-weight (within a range of 1 gram) from the age of three weeks on June 21, 1914, average body-weight 23.6 grams, for seven weeks to the age of ten weeks on August 6, 1914, at which time the average gross body-weight was 23.8 grams. The average daily food-supply of whole wheat (Graham) bread soaked in whole milk for the seven consecutive weeks of the experiment was as follows: 5.1, 3.9, 3.7, 3.5, 3.3, 2.7, 2.7 grams. Similarly, six rats of litter No. 13, average weight 23.1 grams at three weeks of age on June 28, 1914, were held at constant weight for seven weeks until August 12, 1914, when at ten weeks of age their average gross weight was 22.6 grams. Their average daily food-supply for the seven consecutive weeks was as follows: 5.3, 5.0, 4.1, 3.9, 3.3, 3.2, 2.9 grams. In all cases water (city supply, from the Mississippi river) was supplied *ad libitum*. The diminishing amount of food necessary for maintenance cannot be explained as due to increasing temperature, as this was fairly constant. Moreover, a similar condition has been found in other litters at all seasons of the year.

As will be shown later, on account of the intensity of the growth-impulse, especially during the earlier periods of inanition, certain growth-changes occur which require the expenditure of energy. It is possible that this energy is supplied by the excess of food above that required for maintenance proper. Another, but less probable, explanation might be that during inanition the food-intake is in some way more economically utilized, a smaller quantity therefore being sufficient for maintenance. In the later stages of inanition, there is probably a decrease in the temperature of the body, which would therefore require less food.

Rats held at constant body-weight from the age of three weeks to ten weeks, while becoming more active as the experiment proceeds, become at the same time less resistant to cold. They may die suddenly if the room temperature is lowered, or even without any apparent cause. Thus up to sixteen weeks, the longest successful period in those beginning at three weeks, it becomes increasingly difficult to maintain them alive at constant body-weight. When the experiment is begun later, the length of the time during which the body-weight can be held constant is considerably increased. Aron ('11) had a similar experience with dogs, finding it necessary after a time to feed sufficiently to increase the initial body-weight somewhat, in order to keep the animals alive. He explains this as due to the gradual exhaustion of available food-substance stored in the various tissues of the body.

At the end of the various age-periods of the experiment, and at the beginning and end for controls, the rats were killed by chloroform and dissected according to the technique described in previous papers (Jackson and Lowrey '12; Jackson '13, '15 c). The parts, systems and organs were carefully weighed, and portions preserved for microscopic examination (to be considered in a later paper).

As heretofore, in calculating the percentage weights, the *net* body-weight (gross weight less intestinal contents) is taken. The percentage weights of the organs are thus slightly higher than if calculated upon the gross body-weight.

The averages given in the various tables are the arithmetical means of the corresponding individual observations. In view of the comparatively small number of observations and the known variability, especially of some of the organs (cf. Jackson '13), the data are insufficient for treatment by statistical methods, and the values are therefore only fair approximations. They are, however, sufficiently accurate to show some of the more obvious and important changes in the young animal held at constant body-weight. It is hoped that they may be useful as preliminary observations, which may lead to further and more extensive investigations of the various individual organs. In general, the amount of variation found is sufficient to necessitate great caution in drawing conclusions from a small number of observations (sometimes upon a single animal), as frequently happens in experimental work.

LENGTHS OF BODY AND TAIL

The body-length is measured from the tip of the nose to the anus, and the tail-length from the anus to the tip of the tail. The measurements were taken immediately after death, the body and tail being extended by very slight tension. Measurements during life are not practicable, although they might be obtained by the use of anesthetics.

In order therefore to discern the changes in the lengths of body and tail while the body-weight is held constant, it was necessary first to determine these measurements on the normal animal. For this purpose, 450 observations (267 males, 183 females) were available, varying from newborn to about 400 grams body-weight. Of these, 277 (130 males, 147 females) were from the Missouri rats described in a previous paper (Jackson '13), and 25 (13 males, 12 females) from Minnesota. For the remaining 148 (124 males, 24 females) observations upon rats from the colony at The Wistar Institute in Philadelphia, I am indebted to Professor Donaldson and Dr. Hatai. A careful examination revealed no essential differences in the relations of body and tail-lengths according to the source of the rats, so they were all combined into a single series.

The general relations of body and tail-lengths are evident from table 3 (not including the Wistar data, in which the age was usually unknown).

The average ratio of tail to body-length in table 3 was obtained by calculating the ratio for each individual separately, and then taking the mean of the individual ratios. The results are thereby somewhat more accurate than would be obtained by simply taking the ratios of the *average* tail and body-lengths, though the difference is not great.

TABLE 3

Changes in lengths of body and tail

AGE	NUMBER OF OBSERVATIONS		AVERAGE NET BODY-WEIGHT: GRAMS		AVERAGE BODY-LENGTH: MM.		AVERAGE TAIL-LENGTH: MM.		AVERAGE RATIO OF TAIL TO BODY-LENGTH		
	male	female	male	female	male	female	male	female	male	female	total
a. Normal rats (full-fed)											
Newborn	32	36	4.8	4.6	48.7	45.5	16.9	17.5	0.35	0.37	0.36
1 week........	23	27	9.6	9.7	64.5	63.3	29.4	31.4	0.46	0.50	0.48
3 weeks.......	22	22	21.7	18.7	90.4	86.1	59.1	57.9	0.65	0.67	0.66
6 weeks.......	21	16	47.4	45.4	128.0	124.0	110.0	110.0	0.86	0.90	0.88
10 weeks......	19	15	128.0	99.4	173.0	160.0	150.0	143.0	0.87	0.89	0.88
5 to 13 mo.....	16	34	177.7	148.0	190.0	183.0	162.0	168.0	0.86	0.90	0.88
b. Rats under experiment (body-weight held constant)											
(from age of)											
3 to 6 weeks...	3	4	22.4	22.1	98.0	94.4	72.3	77.4	0.74	0.82	0.78
3 to 8 weeks...	1	2	18.1	21.2	94.5	92.0	78.0	80.0	0.83	0.87	0.84
3 to 10 weeks.	8	14	24.7	23.3	105.2	99.6	86.2	83.9	0.82	0.85	0.84
3 to 13 weeks.	0	1		25.5		91.0		85.0		0.93	0.93
3 to 16 weeks.	0	1		26.0		90.0		95.0		1.06	1.06
6 to 32 weeks.	1	1	43.7	50.5	115.0	115.0	105.0	105.0	0.91	0.91	0.91
10 to 35 weeks	2	1	79.6	74.2	140.0	135.0	117.0	115.0	0.84	0.85	0.85

Two facts concerning the relative length of the tail in the normal albino rat are apparent from table 3 a. In the first place, it is evident that at all ages the ratio of the tail-length to the body-length is slightly greater in the female than in the male; that is, the female is relatively long-tailed. By a comparison of the ratios, it appears that the tail of the female on the average is about 4 or 5 per cent longer.

In the second place, it appears that the ratio of tail-length to body-length increases in the normal rat from an average of about 0.36 at birth to 0.48 at one week, 0.66 at three weeks, and 0.88 from the age of six weeks upward. That is, the tail becomes progressively relatively longer, being relatively more than twice as long in the adult as at birth. The acceleration of the tail-growth at such a later period may be cited as an instance of the general law of cranio-caudal progression in development (Jackson '09).

Turning now to the tail-ratio in the rats held at constant body-weight, as shown in table 3 b, and also indicated in the chart in figure 2, it is clear that in rats beginning at three weeks of age there has been a very decided increase in the tail-ratio. The tail at this age evidently continues to elongate, even though the body-weight has been held constant, so that the tail-ratio approaches (although it does not usually quite reach) the normal ratio for rats of corresponding age under normal conditions of growth.

If we compare the absolute lengths of tail and body in the normal rat at three weeks (table 3 a) with those in rats held at constant body-weight from the age of three weeks to the age of six, eight, ten, thirteen and sixteen weeks, it appears that there has also been a slight increase in the absolute length of the body. The increase in tail-length is considerably greater, however, so the tail-ratio increases as above stated.

The larger number of rats were held constant from the age of three to the age of ten weeks. Since the average body-weight for the normal series at three weeks is slightly lower than that of the series held constant to ten weeks of age, I have obtained a new normal series of higher body-weight for direct comparison

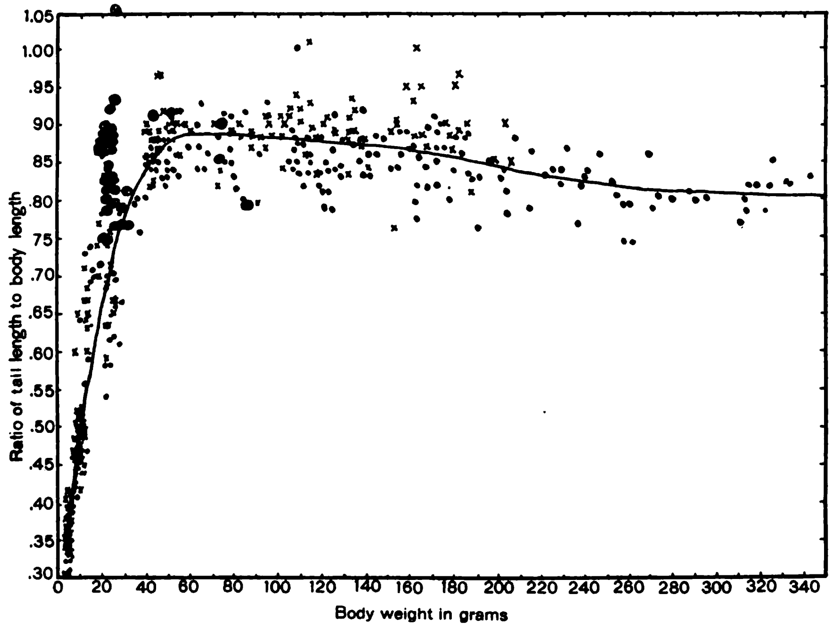

Fig. 2 Chart showing the change in the ratio of tail-length to body (nose-anus) length in the albino rat from birth to maturity. Males are indicated by rounded dots, females by X. The individuals held at constant body-weight during the experiment are encircled. The curve is drawn through points representing the means for the normal individuals at various periods, sexes combined.

(by dropping out the lighter rats of the series given in table 3 a). Thus in sixteen normal males aged three weeks, average net body-weight is 24.2 grams, the average body-length is 94.5 mm. and tail-length is 61.6 mm. (compared with 105.2 mm. body-length and 86.2 mm. tail-length in the 3 to 10 weeks experiment). Similarly, in eight normal females at three weeks, average net body-weight 23.2 grams, the average body-length is 91.1 mm., and tail-length is 63.4 mm. (compared with 99.6 mm. body-length and 83.9 mm. tail-length in the 3 to 10 weeks experiment). In other words, while the body has increased about one-tenth in length, the tail has increased over one-third, the body-weight being held constant from the age of three to ten weeks.

In order to show more clearly this change in the ratio of tail-length to body-length, the individual ratios corresponding to the 450 observations on rats from all sources (including the 148 Wistar rats) were plotted according to body-weight in figure 2. As the sexes are distinguished in the entries, it is evident that the females tend to have a higher tail-ratio. The curve has been drawn through the averages at various periods (sexes combined). It is smoothed free hand, as the labor of constructing the curve more accurately by mathematical methods did not seem justified.

Special attention is called to one apparent discrepancy between the curve in figure 2 and the data in table 3 a. In the latter, it appears that the tail reaches at six weeks an apparent maximum ratio of 0.88 (sexes combined), which is maintained nearly constant at succeeding periods. In the chart, however, it is seen that in rats above 300 grams the average ratio drops to nearly 0.80. These heavy rats are nearly all from the Wistar series, and are all males. It is therefore evident that the drop in the tail-ratio curve is in part due to the fact that no females are included in the higher body-weights. Even taking this into account, however, there is still a decrease in the tail-ratio of the male from a maximum of about 0.86 or 0.87 in rats between 50 and 200 grams body-weight, to nearly 0.80 in rats above 250 grams. (In 7 male rats between 350 and 400 grams, not shown in figure 2, the ratios were slightly above the 0.80 line, the average being 0.81).[2]

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[2] Since the completion of the present paper, I have received, through the courtesy of Professor Donaldson, a manuscript copy of reference tables compiled at The Wistar Institute by formulas for various measurements of the albino rat. These include the body-lengths and tail-lengths, by sexes, from newborn to adult. From these data I have calculated the tail-ratios and find the result in general agreement with the curve shown in figure 2. The tail-ratios calculated from the Wistar tables are somewhat lower, corresponding to body-weights from 30 to 100 grams, however. They also increase steadily, so that at body-weights above 200 grams they lie slightly above the curve in figure 2. The tail-ratio according to the Wistar tables is about 4 per cent higher in the female throughout, when the sexes of equal body-length or body-weight are compared. In rats above 300 grams body-weight, the tail-ratio is about 0.86 in the male and 0.89 in the female.

The two observations on rats held constant from the age of three weeks to the ages of thirteen and sixteen weeks would seem to indicate a continuation of the process of elongation of the tail, even beyond the normal ratio at corresponding age, but the number of observations is too small for definite conclusion.

The number of animals beginning the experiment at later ages, body-weight constant from age of six to age of thirty-two weeks (2) and from ten to thirty-five weeks (3), is also too small to draw any very positive conclusions. However, so far as they go, they indicate (table 3 b; fig. 2) that *between the ages of six and thirty-five weeks there is no marked change in the tail-ratio of rats held at constant body-weight.*

It will be observed that also in the normal rats between six and thirty-five weeks of age there is no apparent change in the tail-ratio, whereas between three and six weeks of age there is normally a decided increase in the tail-ratio. The lengths of the body and tail are of course determined primarily by the growth of the skeleton. I would therefore interpret the results concerning the lengths of tail and body as follows. In young, growing rats held at constant body-weight, the body and tail tend to increase so as to assume the normal ratio at corresponding ages. This is due to the fact that, as will appear later, the skeleton continues to grow in a normal manner (though at a reduced rate) in animals held at constant body-weight.

There is another possible factor in causing the increased tail-ratio in young, growing rats, which may also apply to the similar relative elongation of the tail found in adult rats (cf. Jackson '15 c). Professor Donaldson points out (in a personal communication) that during inanition there may be an arching of the spinal column, producing an actual shortening of the body-length. Such an arching actually does occur, and may be noted especially in young rats during chronic inanition. It is well shown in the stunted rats photographed in figure 1. Of course the greater part of this longitudinal curvature of the spinal column is eliminated by the slight tension exerted in order to straighten out the body when it is measured after death. But it is still quite possible that this does not entirely eliminate the shortening of the column. In any event, however, this is probably a factor of minor importance in altering the tail-ratio during inanition.

Hatai ('08) in a series of five 'stunted' rats in which growth had been retarded (but not stopped) by a diet of starch-mixtures from age of 30 days up to from 127 to 215 days (the final body-weight being from 70.9 to 113.7 grams) finds the average tail-ratio 0.75 as compared with about 0.82 in controls. He notes that:

> The most conspicuous external differences between normal and stunted rats as shown by the stunted rats are in the length of the body and of the tail, both of which were considerably reduced with respect to the body-weight. This peculiar difference, as is seen from the table, holds true in every case. Further, the ratio between the length of the body and that of the tail is considerably less in the stunted rats than in the control rats. . . . Underfeeding therefore produces short tailed individuals.

Recently, however, Dr. Hatai (in a personal communication) states that in other inanition experiments he has obtained different results, and that "rats either grown or kept in a state of chronic inanition (starch feeding, lipoid-free ration and wheat embryo feeding) give a longer tail" in agreement with my results.

Morgulis ('11) in the salamander Diemyctylus found a relatively greater shrinkage in the tail than in the body during inanition; while Harms ('09) found the converse to be true in Triton.

HEAD

The head (table 4; fig. 3) at three weeks normally forms an average of 22.5 per cent of the body, the average net body-weight being 21.2 grams. In the 11 controls at three weeks, the body-weight (24.6 grams) is slightly higher, and the corresponding relative head-weight, 20.6 per cent, somewhat lower. In the rats held constant from the age of three weeks to the ages of six and eight weeks, the average percentage of the head (21.6 per cent and 23.9 per cent) is higher than that of the controls. But the average body-weight in these groups is lower, more nearly the normal above cited, so it is doubtful whether there is any actual increase in the head-weight (either relative or absolute) during the experiment. The larger ten weeks group, however, is nearly equal to the controls in average body-weight, and shows an apparent increase in head-weight from an average of 5.01 to 5.34 grams, or from 20.6 per cent to 22.7 per cent (table 4; fig. 3). In any event, however, the increase in the head-weight is slight, and is not apparent in the two rats held constant from three to thirteen and sixteen weeks (average of the two is 20.6 per cent). On the other hand, there appears to be a slight increase in the weight of the head in the rats held constant from six to thirty-two weeks (15.2 to 17.7 per cent), and from ten to thirty-five (12 to 14.0 per cent).

On the whole, therefore, the evidence would appear to indicate that in young rats held at constant body-weight for considerable periods of time there is a slight increase in the weight of the head. This is probably due to the increase in skeletal weight, which in the head probably overbalances the decrease in the weight of the

TABLE 4

The head; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE) PER CENT
Normal head at 3 weeks (Jackson '13)............	24	21.2	4.60 (3.44– 5.85)	22.5 (16.3–27.3)
Controls at 3 weeks................................	11	24.5	5.01 (4.70– 5.90)	20.6 (18.3–23.0)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.4	4.83 (4.50– 5.10)	21.6 (19.9–23.3)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	3	20.2	4.73 (4.60– 4.90)	23.9 (19.9–26.0)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	22	23.8	5.34 (4.60– 6.50)	22.7 (20.4–26.2)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25.5	5.00	19.6
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	5.50	21.6
Normal at 6 weeks (Jackson '13)..................	42	50.0	7.40 (6.30–9.40)	15.2 (11.3–18.0)
Controls at 6 weeks................................	2	42.4	6.40 (6.20– 6.60)	15.0 (14.6–15.5)
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	8.30 (8.20– 8.50)	17.7 (16.8–18.6)
Controls at 10 weeks...............................	6	134.0	13.30 (11.10–14.70)	10.1 (8.6–11.7)
Body-weight constant 25 weeks (age of 10 to 35 weeks (normal head for corresponding body-weight of 85 grams forms about 12.0 per cent.............	1	85.5	12.10	14.0
Controls at 32 and 35 weeks........................	6	189.5	20.30 (17.40–26.30)	10.7 (9.8–11.4)

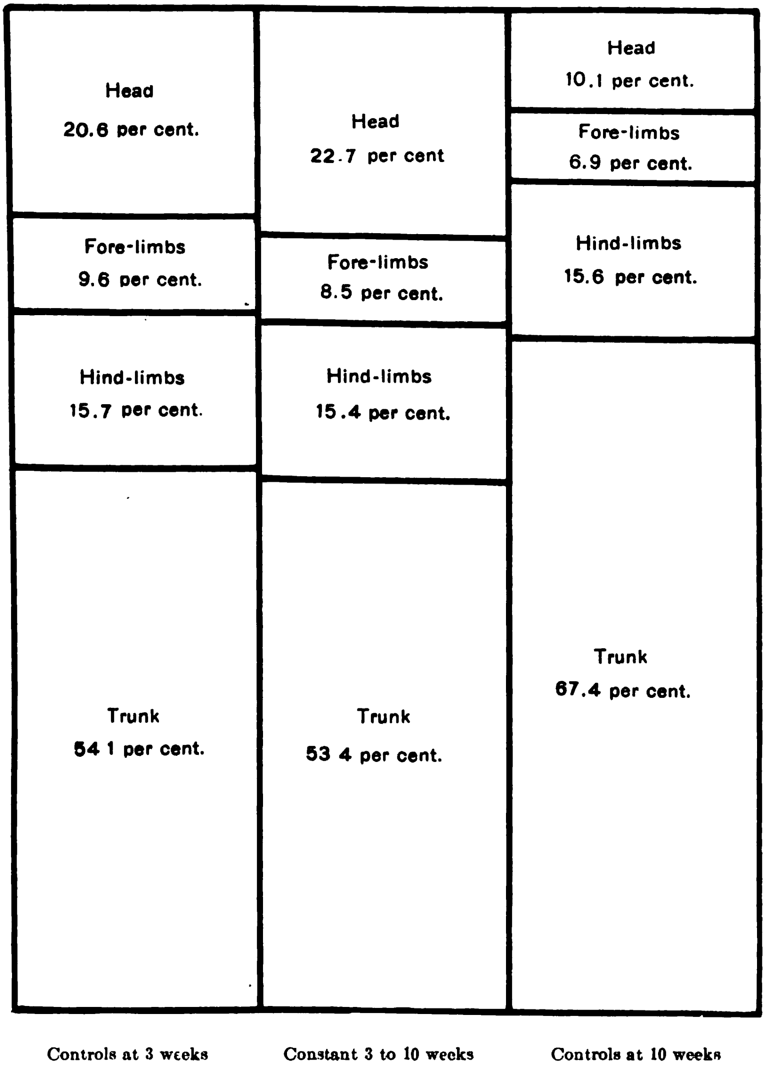

Fig. 3 Diagram representing the average relative (percentage) weights of parts of the body (head, extremities and trunk) in albino rats held at constant body-weight from the age of three to ten weeks, and in controls at three and at ten weeks of age.

integument; although, as will be shown later, the average loss in the integument of the *entire body* is relatively slightly greater than the corresponding skeletal increase (fig. 4).

In both acute and chronic inanition in adult rats (Jackson '15 a, '15 c) the head increases very markedly in relative weight, the loss in absolute weight being but very slight in comparison with the loss in weight of the entire body.

EXTREMITIES AND TRUNK

The extremities (table 5; fig. 3) were separated at the shoulder-joint and hip-joint, respectively. There is apparently a slight decrease in the relative weight of the fore-limbs in the young rats held at constant body-weight from the age of three weeks to six, eight, ten, thirteen and sixteen weeks of age. In the case of the rats held constant from three to ten weeks, the apparent decrease is from an average of 9.6 per cent to 8.5 per cent. On account of the small number of observations, however, and the difficulty in separating the limbs (especially the integument) in an absolutely uniform manner, the slight apparent decrease is of doubtful significance.

In the case of the hind-limbs, there is likewise an apparent indication of a slight decrease, but even less marked than in the fore-limbs. The apparent average decrease from 15.7 per cent to 15.4 per cent of the body-weight in the largest group (three to ten weeks, is well within the limits of experimental error.

On the whole, therefore, it is doubtful whether there is any distinct and significant change in the weights of the extremities in young rats held at constant body-weight for considerable periods. A slight loss, however, might be accounted for by the slightly greater loss in the integument (as compared with the gain by the skeleton); especially since the integument of the limbs probably forms a relatively larger part of the limbs than the whole integument does of the whole body.

The trunk was not weighed directly, but its weight was calculated by subtracting from the net body-weight the weight of the head and extremities. From what has been said con-

TABLE 5

The extremities; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE) PER CENT
a. Fore-limbs				
Normal at 3 weeks (Jackson and Lowrey)........	4	25.5	2.36	9.3 (7.4-10.90)
Controls at 3 weeks..............................	6	23.8	2.29 (2.00-2.51)	9.6 (8.7-10.60)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	4	23.1	1.68 (1.50-1.90)	7.3 (6.7- 8 30)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	1	24.6	1.70	6.9
Body-weight constant 7 weeks (age of 3 to 10 weeks)	8	22.3	1.84 (1.60-2.20)	8.5 (7.4- 9.50)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25.5	1.80	7.1
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	1.80	6.9
Normal at 6 weeks (Jackson and Lowrey).........	4	79.2	5.30	6.7 (5.9- 8 22)
Controls at 6 weeks..............................	1	42.3	3.00	7.1
Controls at 10 weeks.............................	2	127.9	8.90 (7.20-10.60)	6.9 (6.3- 7 60)
Normal at 10 weeks (Jackson and Lowrey).......	3	141.9	7.60	5.3 (5.21-5.50)
b. Hind-limbs				
Normal at 3 weeks (Jackson and Lowrey)	4	25.5	3.80	14.9 (14.3-15.40)
Controls at 3 weeks..............................	6	23.8	3.75 (3.20-4.13)	15.7 (14.3-15.90)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	4	23.1	2.97 (2.55-3.60)	12.8 (11.6-15.10)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	1	24.6	3.40	13.8
Body-weight constant 7 weeks (age of 3 to 10 weeks)	8	22.3	3.38 (3.00-3.80)	15.4 (14.3-15.90)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25 5	3.20	12.4
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	3.20	12.6
Normal at 6 weeks (Jackson and Lowrey).........	4	79.2	11.80	14.9 (14.6-15.40)
Controls at 6 weeks..............................	1	42.3	5.60	13.2
Controls at 10 weeks.............................	2	127.9	19.90	15.6 (15.4-15 80)
Normal at 10 weeks (Jackson and Lowrey)........	3	141.9	22.10	15.6 (14.5-16.50)

TABLE 6

The integument; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE) PER CENT
Normal integument at 3 weeks (Jackson and Lowrey).................................	13	24.8	5.55	22.4 (18.7 -29.2)
Controls at 3 weeks..............................	10	25.1	5.59 (3.86- 6.36)	21.9 (18.4 -25.2)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	8	22.2	2.81 (2.21- 3.21)	12.5 (9.82-14.1)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	3	20.2	2.78 (2.27- 3.30)	13.8 (12.6 -15.5)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	22	23.8	3.41 (2.63- 4.51)	14.5 (12.3 -17 5)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25.5	3.50	13.7
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	3.30	12.7
Normal at 6 weeks (Jackson and Lowrey).........	14	64.4	13.46	20.9 (16.7 -25.9)
Controls at 6 weeks..............................	2	42.4	8.04 (8.00- 8.08)	17.0 (15.1 -19.0)
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	6.15 (5.70- 6.60)	13.0 (12.9 -13.1)
Normal at 10 weeks (Jackson and Lowrey)........	10	131.0	24.50	18.7 (15.6 -22.3)
Controls at 10 weeks.............................	6	134.0	26.70 (19.40-32.10)	20.0 (17.8 -22.0)
Bodyweight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	14 00 (12.30-17.20)	17.9 (16.6 -20.1)
Controls at 32 and 35 weeks.......................	6	189 5	38 40 (28.10-55.20)	20.1 (18.0 -23 2)

cerning the head and extremities, it follows that there cannot be much change in the trunk-weight, since the probable slight increase in the weight of the head is off-set by a slight decrease in the extremities. In the largest group, however, held at constant body-weight from three to ten weeks of age (fig. 3), the trunk would apparently decrease from an average of 54.1 per cent to 53.4 per cent. This apparent change is so slight as to be (probably) insignificant.

The results concerning the parts of the body therefore fail to indicate any decided change of proportional weights in young animals held for considerable periods at constant body-weight. There is apparently a very small increase in the head, counterbalanced by a corresponding decrease in the trunk and extremities, but the change is so slight as to be of doubtful significance. During inanition in adult rats, there is apparently a relative increase in both head and extremities, counterbalanced by a relative decrease in the trunk (Jackson '15 a, ' 15c).

INTEGUMENT

In the rats held at constant body-weight from the age of three weeks to six, eight, ten, thirteen and sixteen weeks, there is a very marked loss in the weight of the integument (including hair and nails; table 6; fig. 4). In the case of the largest (three to ten weeks) group, the decrease is from an average of 21.9 per cent to 14.5 per cent of the body-weight. In terms of absolute weight, the decrease is from 5.30 grams (5.59 grams, less correction on account of difference in body-weight, which averages 25.1 grams at three weeks and 23.8 grams at ten weeks) to 3.41 grams, a decrease of about 36 per cent. The decrease would appear slightly greater if the difference in relative weight of the integument for different initial body-weights were taken into account. There is apparently even greater loss at the other ages. It would appear that this loss (which is perhaps chiefly a loss of fat) occurs rather early, as at six weeks (body-weight held constant three weeks) the loss is as great as at subsequent and longer periods.

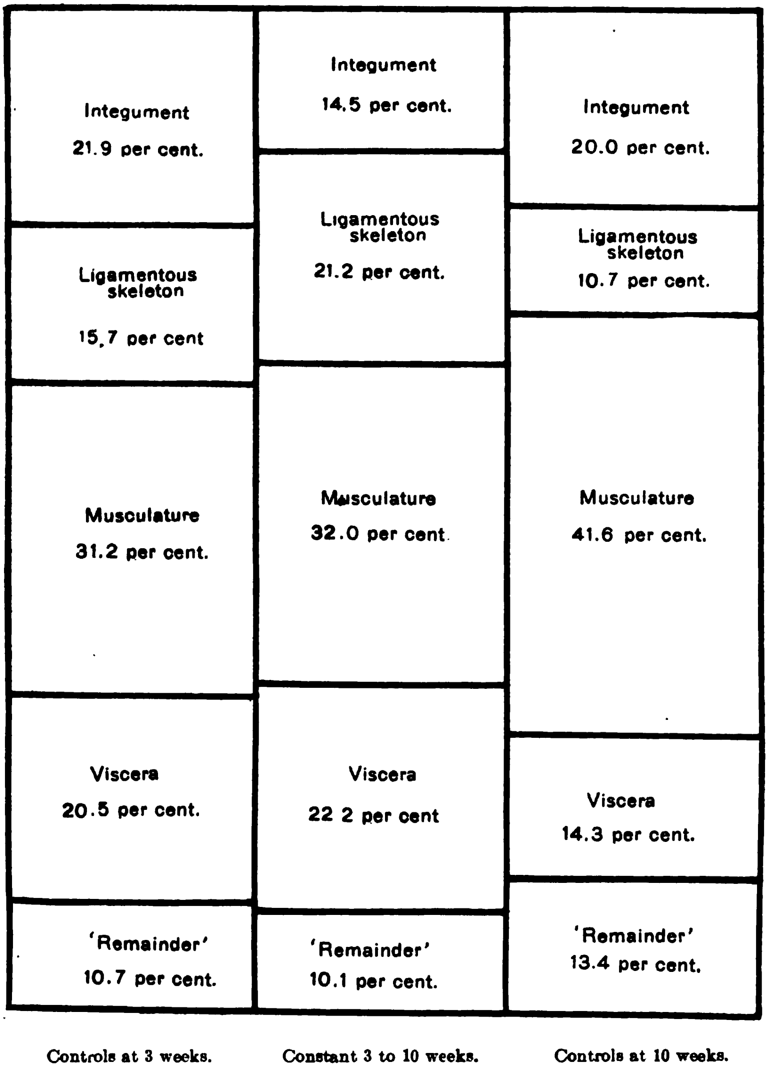

Fig. 4 Diagram representing the average relative (percentage) weights of the various systems (integument, skeleton, musculature, viscera and 'remainder') in albino rats held at constant body-weight from the age of three to ten weeks, and in controls at three and at ten weeks of age.

In the rats experimented upon at later and longer periods (ages of six to thirty-two weeks and ten to thirty-five weeks) there is also a marked loss in the weight of the skin, though apparently not so great as at the earlier periods.

This loss in the weight of the integument is in striking contrast with the results of inanition in adult rats (Jackson '15 a, '15c). Here the loss is very nearly proportional to that of the whole body, so the integument nearly maintains its relative (percentage) weight.

From his experiments upon young dogs held at constant body-weight, Aron ('11, p. 29) states that: "The skin shows a slightly higher percentage of the body-weight in those animals kept at a constant weight than in the normal, control dogs. These figures indicate that, while the (body) weight was constant, the skin increased very slightly in weight." The figures cited show the skin in animals held at nearly constant body-weight to form (in four cases) 12.2 to 14.6 per cent of the body-weight, whereas in three corresponding full-fed controls the skin formed 11.2 to 13.0 per cent. Aron, however, overlooks the fact that he is making his comparison with controls at the *end* of the experiment. In order to judge what changes have taken placed during the experiment, the comparison must be with normal control animals killed at the *beginning* of the experiment. Aron records but one case which can be used for this purpose. His Dog D (table 13, Experiment IV) killed at the age of 40 days, the beginning of the experiment, with body-weight of 1985 grams shows a skin-weight of 320 grams, or about 16.1 per cent of the body-weight. From Aron's own data, therefore, I would reach the opposite conclusion, viz., that in young dogs held at constant body-weight, the skin suffers a marked loss in weight. This would agree with my results on rats.

SKELETON

The skeleton (table 7; fig. 4) was prepared in three ways. The bones, together with the cartilages, periosteum and ligaments, constitute the 'ligamentous skeleton' (table 7 a). The bones and cartilages, after removal of the periosteum and ligaments by immersion for about one hour in 1 per cent aqueous 'gold dust 'solution at 90°C., constitute the 'cartilaginous skeleton' (table 7 b). Finally the cartilaginous skeleton dried in an oven at 95°C. to constant weight constitutes the 'dry skeleton' (table 7 c).

An examination of the weight of the ligamentous skeleton (table 7 a) reveals the striking fact that while the body-weight

TABLE 7

The skeleton; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE) PER CENT
a. Ligamentous skeleton				
Normal at 3 weeks (Jackson and Lowrey)	13	24.8	4.12	16.6 (13.1 -21.10)
Controls at 3 weeks	12	24.5	3.90 (3.100- 5.46)	15.7 (13.5 -17.00)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	8	22.2	4.04 (3.140- 5.50)	18.0 (14.6 -23.90)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	3	20.2	4.74 (4.290- 5.50)	23.7 (22.3 -24.90)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	22	23.8	4.98 (3.700- 7.14)	21.2 (17.7 -24.60)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25.5	4.80	18.8
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	5.20	20.0
Normal at 6 weeks (Jackson and Lowrey)	14	64.4	9.02	14.0 (10.5 -20.10)
Controls at 6 weeks	2	42.4	5.48 (4.750- 6.20)	13.0 (11.2 -14.70)
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	7.20 (6.800- 7.60)	15.4 (15.1 -15.60)
Normal at 10 weeks (Jackson and Lowrey)	10	131.0	15.30	11.7 (10.0 -12.90)
Controls at 10 weeks	6	134.0	14.00 (11.400-17.60)	10.7 (8.5 -14.10)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	10.30 (9.400-11.60)	13.3 (12.7 -13.70)
Controls at 32 and 35 weeks	6	189.5	18.60 (15.600-24.30)	9.8 (8.8 -11.20)
b. Cartilaginous skeleton (Fresh)				
Controls at 3 weeks	6	22.9	2.60 (2.120- 3.00)	11.4 (9.0 -12.90)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	3	22.9	3.56 (2.930- 4.04)	15.5 (13.3 -17.00)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	1	24.6	4.50	18.3
Body-weight constant 7 weeks (age of 3 to 10 weeks)	8	22.4	3.16 (2.420- 4.00)	14.6 (11.9 -17.10)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25.5	4.00	15.7
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	3.96	15.2
Controls at 10 weeks	1	115.0	6.40	5.6
c. Dry skeleton (cartilaginous)				
Controls at 3 weeks	5	23.4	0.804 (0.710-0.964)	3.43 (3.17- 4.03)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	3	21.9	1.091 (1.033-1.172)	4.98 (4.70- 5.13)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	1	24.6	1.351	4.59
Body-weight constant 7 weeks (age of 3 to 10 weeks)	9	22.2	1.285 (1.076-1.485)	5.84 (4.76- 7.00)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25.5	1.068	6.31
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	1.744	6.71
Control at 10 weeks	1	115.0	3.420	2.97

is held constant the skeleton continues to increase in weight to a marked degree. In the rats beginning at three weeks, there is an increase in the relative weight of the ligamentous skeleton from 15.7 per cent of the body to 18.0 per cent at six weeks and to an apparent maximum of 23.7 per cent at eight weeks. This latter is probably an exceptional figure, as in the largest group, at ten weeks, the average is 21.2 per cent (fig. 4). This corresponds to an increase from an absolute weight of 3.90 to 4.98 grams, an increase of about 28 per cent (or slightly more, if correction be made for the difference in body-weight, average 24.5 grams at three weeks and 23.8 grams at ten weeks). The two cases carried to thirteen and sixteen weeks, respectively, show a slightly smaller relative increase. The rats used at later and longer periods (ages of six to thirty-two weeks and ten to thirty-five weeks) also show a considerable increase in the skeleton, though relatively less than those beginning at the earlier period.

The data for the cartilaginous skeleton (table 7 b) similarly show a marked increase in rats held at constant body-weight for various periods beginning at three weeks of age. The figures for the largest group (three to ten weeks) indicate an increase from 11.4 per cent to 14.6 per cent of the body. In terms of absolute weight, the increase is from an average of 2.60 grams (body-weight 22.9 grams) to 3.16 grams (body-weight 22.4 grams), an increase of about one-fourth. Subtracting the percentage weights of the cartilaginous skeleton from the corresponding ligamentous skeleton, there is (for the three to ten weeks group) an evident increase of the ligaments and periosteum from 4.3 per cent to 6.6 per cent of the net body-weight. This would indicate that the ligamentous component of the skeleton shares in the marked growth during constant body-weight.

Professor Donaldson (in a personal communication) has kindly supplied a series of observations showing that the cartilaginous skeleton in the normal rat changes from a relative weight of about 10 per cent of the body at 20 grams to 7.5 per cent at 50 grams, 7 per cent at 100 grams and 6.7 per cent in rats above

200 grams. These weights, however, do not include the intervertebral discs.

The data for the dried cartilaginous skeleton (table 7 c) indicate an even greater increase in the dry skeleton of the rats held at constant body-weight. Thus in rats beginning at three weeks the dry substance increases from 3.43 per cent of the body weight to 4.98 per cent at six weeks of age, 5.49 per cent at eight weeks, 5.84 per cent at ten weeks, 6.31 per cent at thirteen weeks and 6.71 per cent at sixteen weeks.

Since the increase in the dry skeleton is relatively greater than that for the (moist) cartilaginous skeleton, it necessarily follows that the skeleton must be losing in percentage of water and gaining in percentage of dry substance. The percentage of dry substance has been calculated for each individual skeleton included in tables 7 a and 7 b, and the averages for each group are as follows: controls at three weeks, 31.4 per cent; constant three to six weeks of age, 33.5 per cent; three to eight weeks, 30.0 per cent; three to ten weeks, 41.7 per cent; three to thirteen weeks, 40.2 per cent; three to sixteen weeks, 44.0 per cent; control at ten weeks, 53.4 per cent.

Lowrey ('13) finds the dry substance of the *ligamentous* skeleton in the normal albino rat to increase from an average of 33.3 per cent at 20 days of age to 39.2 per cent at six weeks, 45.9 per cent at ten weeks, 50.4 per cent at five months and 52.6 per cent at one year.

From the foregoing it is evident that in rats held at constant body-weight beginning at three weeks, the growing cartilaginous skeleton steadily increases its percentage of dry substance. Thus it tends to change the proportions of water and dry substance as during normal growth. The percentage of dry substance does not increase so rapidly with age as during normal growth, however, but lags behind corresponding to the retardation in absolute growth. During inanition in adult rats, on the contrary, there is a relative decrease in the dry substance, and an increase in water-content (Jackson '15 c).

It has already been noted in a previous section ("Lengths of body and tail") that in the rats held at constant body-weight beginning at the age of three weeks there is an increase in the lengths of both body and tail. The latter increases more rapidly. however, so that it tends to assume the tail-ratio found in normal rats of corresponding age. This indicates that the skeleton not only continues to grow (though at a reduced rate) while the body-weight is held constant, but also tends to *grow in a normal manner*, so as to produce the normal ratio of tail-length and body-length. The preceding paragraphs have shown that the increased growth of skeleton affects the ligamentous as well as the cartilaginous and bony components, and that the chemical composition (percentages of water and dry substance) also changes in a manner tending to assume the normal.

The question naturally arises as to whether the skeletal growth during constant body-weight is merely a growth in mass, or is associated with the normal process of *differentiation*. During the present investigation a few observations have been made upon the development of the normal skeleton, indicating some of the more obvious changes during the age-periods of the rats under experiment, especially between the ages of three and ten weeks. While a detailed study of the developmental changes in the skeleton is reserved for a separate paper, some preliminary conclusions may be noted here.

In skeletons of rats held at constant body-weight from the age of three to the age of ten weeks, the appearance and fusion of certain epiphyses may be noted as in the normal animal during this period, although in most cases the process appears to be retarded somewhat. The following examples may be cited. In the normal skeleton at three weeks of age, the epiphyses at the ends of the vertebral bodies have not appeared; the epiphysis at the lower end of the humerus is well developed, but not fused with the shaft; the maxilla and mandible present each two molars (on each side), with no visible trace of a third. In the normal skeleton at ten weeks, the epiphyses at the ends of the vertebral bodies have appeared, and most of them have united with the corresponding bones; the epiphysis at the lower end of the humerus is firmly fused with the shaft; well developed third molar teeth have appeared, both in the maxilla and in the mandible. In the skeleton of a rat held at constant body-weight from three to ten weeks of age, most of the epiphyses of the vertebral bodies have appeared, and they usually have united at one end of each bone; the lower epiphysis of the humerus is firmly united with the shaft, as normally at ten weeks; well-developed third molars have appeared, as normally at ten weeks. In a rat held at constant body-weight from age of three to sixteen weeks, the skeletal differentiation was more advanced, corresponding at least to the stage reached normally at ten weeks, and in some respects perhaps even beyond it.

These observations will suffice to establish the fact that the skeletal growth during constant body-weight is accompanied by normal developmental changes, as well as changes in chemical composition (percentage of water). In other words, we find not only increase in mass but growth and differentiation apparently normal in character, though somewhat retarded in rate. These skeletal characters therefore tend to correlation with age, although influenced also by the general body-weight.

The remarkable fact that the skeleton continues to grow while the body-weight is held constant was apparently first observed by Waters ('08) who found that calves previously well nourished will continue to increase in height and in width of hip for a considerable time, even when increase of body-weight is prevented by under-feeding. He remarks ('08 b, p. 9):

> Apparently the animal organism is capable of drawing upon its reserve for the purposes of sustaining the growth process, for a considerable time and to a considerable extent. Our experiments indicate that after the reserve is drawn upon to a certain extent to support growth, the process ceases and there is no further increase in height or in length of bone. From this point on, the animal's chief business seems to be to sustain life. This law applies to animals on a stationary live weight as well as to those being fed so that the live weight is steadily declining, and indeed to those whose ration, while above maintenance, and causing a gain in live weight, is less than the normal growth rate of the individual. Such an animal will, while gaining in weight, get thinner, because it is drawing upon its reserve to supplement the ration in its effort to grow at a normal rate.

Aron ('11) experimented with dogs to determine the effect of a restricted amount of food upon young, growing animals. He found that in spite of constant live weight, the animals continued to increase in length and height for three to five months. Thereupon the emaciated animals became weaker and died unless the amount of food was somewhat increased. From a comparative study of nine bones (the entire skeleton was not measured), Aron concludes that the skeleton during constant body-weight increases in mass and also changes in chemical composition (increase in water-content and protein (?); decrease in fat). The results of this very interesting investigation, while sufficient to establish the continued growth of the skeleton, would be more conclusive if the number of observations were larger, with an adequate number of controls at the beginning and at the end of the experiment. In a recent paper, Aron ('14) records a few observations indicating that malnutrition in children retards growth in length less than body-weight; so that the body may continue to increase in length while the body-weight is at a standstill, or even slightly decreasing. Thus the strong growth tendency of the skeleton during bare maintenance of the body-weight is manifest in the human species, as well as in the calves, dogs and rats.

MUSCULATURE

Although the musculature (table 8; fig. 4) in the normal rat at three weeks averages 26.9 per cent of the body, according to Jackson and Lowrey ('12), the controls in the present series gave a somewhat higher amount, the average being 31.2 per cent. As shown in table 8, the musculature in the controls of the present series also averaged slightly higher than the normal according to Jackson and Lowrey at six and ten weeks. In rats held at constant body-weight from the age of three weeks to six, ten and thirteen weeks, the musculature appears relatively very slightly higher than in the controls at three weeks. The apparent increase from three to ten weeks is from 7.40 grams (7.81 grams, less correction corresponding to the smaller body-weight at 10 weeks) to 7.62 grams, or an increase of 3.0 per cent in absolute weight. In the three to sixteen weeks experiment,

TABLE 8

The musculature; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson and Lowrey)	13	24.8	6.67	26.9 (20.1-30.2)
Controls at 3 weeks	10	25.1	7.81 (6.90-9.82)	31.2 (29.5-35.3)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	8	22.2	7.04 (5.51-8.05)	31.8 (25.1-34.7)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	3	20.2	6.46 (5.68-7.70)	32.1 (31.3-33.2)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	22	23.8	7.62 (4.58-10.87)	32.0 (24.8-36.4)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25.5	7.90	31.0
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	7.50	29.4
Normal at 6 weeks (Jackson and Lowrey)	14	64.4	21.10	32.7 (26.1-35.3)
Controls at 6 weeks	2	42.4	14.90 (14.85-15.0)	35.3 (35.0-35.6)
Body-weight constant 26 weeks (age of 6 to32 weeks)	2	47.1	16.90 (15.30-18.5)	35.7 (34.8-36.6)
Normal at 10 weeks (Jackson and Lowrey)	10	134.0	55.10	41.1 (37.4-49.1)
Controls at 10 weeks	6	135.0	55.80 (44.00-69.2)	41.6 (39.3-44.5)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	31.20 (30.10-33.9)	40.1 (39.7-40.7)
Controls at 32 and 35 weeks	6	189.5	81.20 (64.80-119.7)	42.6 (39.0-50.3)

TABLE 9

Viscera and remainder; average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	RELATIVE WEIGHT OF VISCERA (AND RANGE) PER CENT	RELATIVE WEIGHT OF 'REMAINDER' (AND RANGE) PER CENT
Normal at 3 weeks (Jackson and Lowrey)	13	24.8	21.3 (20.1-24.8)	12.9 (4.0-19.9)
Controls at 3 weeks	10	25.1	20.5 (17.9-24.8)	10.5 (2.6-15.6)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	25.0 (20.7-29.2)	13.6 (7.1-21.6)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	26.8 (25.0-28.5)	2.3 (1.9-2.7)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	22.2 (19.4-26.0)	10.0 (2.5-16.9)
Normal at 6 weeks (Jackson and Lowrey)	14	64.5	20.4 (18.4-22.9)	12.0 (6.5-17.1)
Controls at 6 weeks	1	42.4	19.6	19.1
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	19.5 (19.1-19.9)	16.4 (16.1-16.7)
Normal at 10 weeks (Jackson and Lowrey)	10	130.5	16.0 (14.9-17.2)	12.5 (1.2-18.4)
Controls at 10 weeks	6	135.0	14.3 (13.0-15.7)	13.6 (9.7-16.4)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	16.3 (16.1-16.8)	12.4 (9.7-13.9)
Controls at 32 and 35 weeks	5	179.8	12.9 (11.9-13.5)	17.0 (13.7-19.5)

the average is slightly lower. In the six to thirty-two weeks experiment, the musculature appears very slightly higher than in the controls at six weeks, while in the ten to thirty-five weeks experiment, the musculature appears slightly lower than in the controls at ten weeks. In the latter case, however, the controls are too heavy for comparison with those under experiment.

In general, it seems clear from the foregoing that in young rats held at constant body-weight the musculature also remains nearly constant in weight, with perhaps a very slight tendency to increase in the majority of cases. In the course of normal growth during this period, the musculature shows a more rapid growth than any other system, increasing from about 27 per cent of the body at three weeks to 41 per cent at ten weeks of age (Jackson and Lowrey). During inanition in adult rats, the musculature loses approximately in proportion to the entire body, slightly less in acute inanition and slightly more in chronic inanition (Jackson '15 c)

Aron ('11) did not weigh the muscles in his experiments on dogs, but infers (p. 29) that: "Only the flesh, muscles and fat of the body remain as the tissues which must have lost during the course of the experiments." From an analysis of samples taken from the leg muscles, he also concludes that "The muscles contained only one-half of the normal amount of solids," the protein being greatly decreased and the water-content increased. Again, however, his comparison is with controls at the *end* rather than the *beginning* of the experiment, so that no conclusion can be drawn as to the changes taking place during the experiment in the animals held at constant body-weight.

VISCERA AND 'REMAINDER'

With the visceral group (table 9; fig. 4) have been included the brain, spinal cord and eyeballs, as well as the thoracic and abdominal viscera. According to Jackson and Lowrey '12, this group decreases from about 21 per cent of the body at three weeks to about 16 per cent at ten weeks of age. This is in fairly close agreement with the controls in the present series, except at ten weeks. In this case the controls are considerably too heavy for direct comparison with the animals under experiment, which accounts for the discrepancy.

In the animals held at constant body-weight from the age of three weeks to the ages of six, eight and ten weeks, the visceral group shows a distinct increase in weight. This is more marked at six and eight than at ten weeks, which perhaps indicates that the viscera may increase in the earlier part of the experiment, and lose weight later. The experiment from six to thirty-two weeks indicates no essential change in the weight of the viscera. From ten to thirty-five weeks there is a slight gain.

On the whole, it may be concluded that during constant body-weight in young albino rats the visceral group as a whole undergoes but little change in weight, with a slight tendency to increase, especially in the earlier periods. As will be seen later, however, the individual viscera differ greatly in their reactions.

Aron ('11) concludes that in young dogs held at nearly constant body-weight the organs in general do not lose weight. On account of the small number of observations, however, and the lack of adequate controls, it is difficult to draw any satisfactory conclusion from his observations upon the viscera.

The 'remainder' is obtained by deducting from the net body-weight the weight of the integument, skeleton, musculature and viscera. It therefore includes loss by evaporation and escape of fluids, as well as a few small unweighed organs and the masses of dissectable fat. The data in table 9 show a considerable variation, as might be expected. On the whole, however, it appears doubtful whether there is any material change in the weight of the 'remainder' in young rats held at constant body-weight for considerable periods. There is undoubtedly a loss in the fat, but this is probably counterbalanced by an increased water-content of the interstitial connective tissues.

BRAIN

The brain (table 10) in eleven controls at three weeks of age averaged 1.282 grams, or 5.31 per cent of the (net) body-weight. This corresponds fairly closely with Donaldson's ('08) figure for the normal rat of corresponding weight. In the rats held at constant body-weight from the age of three to six and eight weeks, there appears (table 10) to be a relative increase in the brain, especially at eight weeks, where it forms 6.62 per cent of the body. In reality, however, this relative increase is only apparent, and due to the fact that these animals began the experiment at a lower body-weight, corresponding to which the brain is relatively heavier. The (net) body-weights of the two rats at eight weeks were respectively 18.1 grams and 17.8 grams. According to Jackson ('13, p. 22), the brain normally reaches its maximum relative weight of about 6.7 per cent of the body when the body-weight is about 15 grams. Thus the final brain-weight of the rats held at constant body-weight from the age of three to eight weeks is almost exactly that to be expected if the brain-weight has remained constant.

TABLE 10

The brain; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Donaldson '08, table 1)	52	25.0*	1.285	5.14
Controls at 3 weeks	11	24.5	1.282 (1.187–1.364)	5.31 (4.14–6.20)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	1.195 (1.035–1.297)	5.44 (4.50–6.33)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	1.183 (1.180–1.186)	6.62 (6.60–6.63)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	1.267 (1.136–1.379)	5.30 (4.38–6.20)
Normal at 6 weeks (Donaldson '08)	42	45.0*	1.441	3.20
Control at 6 weeks	1	42.4	1.373	3.23
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	1.478 (1.459–1.497)	3.14 (2.96–3.32)
Normal at 10 weeks (Donaldson '08)	34	75.0*	1.559	2.08
Controls at 10 weeks†	6	134.0	1.579 (1.512–1.636)	1.21 (0.96–1.44)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	1.646 (1.603–1.723)	2.12 (2.02–2.18)
Controls at 32 and 35 weeks	6	189.5	1.777 (1.657–1.890)	0.97 (0.78–1.24)

* Gross body-weight.

† Body-weight of controls at 10 weeks too high for comparison.

The brain weight has also apparently remained nearly constant in the large group held at constant body-weight from three to ten weeks of age. The average absolute weight is slightly less, but almost in correspondence with the body-weight, so that the average *relative* weight, 5.30 per cent, is nearly identical with that of the controls at three weeks, the beginning of the experiment. In terms of absolute weight, there is a very slight apparent decrease from 1.274 grams (1.282 grams, less correction[3] for difference in body-weight, which averages 24.5 grams in the three weeks controls and 24.0 grams at ten weeks) to 1.267 grams, a decrease of about 0.5 per cent in absolute weight.

In the series held at constant body-weight from the age of six to thirty-two weeks, there is an apparent slight decrease in the brain from about 3.23 per cent to 3.14 per cent of the body, and in the ten to thirty-five weeks series a slight increase (from 2.08 to 2.12 per cent). Considering the small number of observations and the normal variation, however, these apparent differences do not appear to be significant. I would, therefore, conclude from the data above cited that there is probably no appreciable change in the weight of the brain in young albino rats held at constant body-weight for considerable periods.

Hatai ('04) experimented with a series of young rats with initial body-weights corresponding roughly to those of mine at the ages of six to ten weeks. By giving an unfavorable diet (starch and beef-fat) their body-weight was reduced on the average about 30 per cent. The brain in these cases had apparently lost in absolute weight, the average loss being about 5 per cent. These results, however, are of course not directly comparable with those in which the body-weight has remained constant.

In a later experiment, Hatai ('08) by underfeeding with unfavorable diet retarded the growth of a series of five rats, beginning at the age of 30 days, so that at 170 days their average weight was only 91.5 grams, while full-fed controls averaged 146.5 grams. By comparison with 'second controls,' younger rats of body-weight similar to the final weight of the stunted series, he found that in the stunted rats the brain-weight was practically identical with that of normal rats of the same body-weight. In other words, the growth in brain-weight had been retarded in the same proportion as the body-weight. On this principle, if the body-weight were retarded so as to permit no growth at all, that is held at constant weight, we should expect practically no increase in weight of the brain. This is in agreement with my results, as above stated.

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[3] It should be noted here as in other cases that the correction for organ-weight is not in exact proportion to the difference in body-weight. Allowance must be made for the change in the *relative* weight of the organ corresponding to the change in body-weight.

More recently Donaldson ('11) has experimented with a larger series (twenty-two litters) of rats held at nearly constant weight (34 grams) from the age of thirty to the age of fifty-one days. In the rats held at constant body-weight, the brain weight averaged 7.7 per cent less than in full-fed controls of the same litters. No direct controls were taken at the *beginning* of the experiment, but from the normal growth formula it is estimated that the initial brain-weight was slightly less than that found in the retarded rats at the end of the experiment. This would indicate an increase of 3.6 per cent in the brain-weight, while the body-weight was held constant. The large number of observations lends weight to this conclusion, although it would be strengthened if direct controls were available at the beginning of the experiment.

It may be noted that if Donaldson's normal (Wistar reference tables) rather than the direct controls be taken as the basis for estimating the initial brain-weight in my three to ten weeks series, the result would indicate a gain similar to that found by Donaldson in his series. On the whole, therefore, we may safely conclude that there is but very slight if any increase in the brain-weight of young albino rats held at constant body-weight for considerable periods of time.

SPINAL CORD

When the relative weights are compared with the controls at the beginning of the experiment (table 11), or with the theoretical normal according to Donaldson ('08) there appears a very decided increase in the spinal cord at all the age-periods during the experiment. Thus while the body-weight has been held constant from the age of three to that of ten weeks, the spinal cord has apparently increased from an average of 0.179 to 0.243 grams, an increase of about 36 per cent (or slightly more if the initial weight be decreased to correct for the difference of body-weight at three weeks, 24.5 grams, and ten weeks, 24.0 grams). This corresponds to an increase from 0.74 per cent to 1.02 per cent of the net body-weight. The increases at the other age-periods are equally striking.

Donaldson ('11) in the experiments previously mentioned also found an increase in the weight of the spinal cord in rats held at body-weight of about 34 grams from the age of thirty days to that of fifty-one days. He does not estimate this increase exactly but from the normal weight of the cord at the beginning of the experiment (cf. Donaldson '08, table 1) the weight must have increased from about 0.223 to 0.2498 grams, an increase of about 10.7 per cent. While this is not so striking as my results (perhaps in part because my experiments covered a longer period of time) it agrees in indicating during constant body-weight a much stronger growth tendency in the spinal cord than in the brain. This is in agreement with the well-known fact that in general the normal post-natal growth of the spinal cord is relatively much more rapid than that of the brain. This growth of the spinal cord is apparently correlated with the increase in trunk-length (Donaldson).

EYEBALLS

An increase even more striking than that of the spinal cord is apparent in the eyeballs (table 12). In rats held at constant body-weight from the age of three weeks, the eyeballs increase from a relative weight of about 0.50 per cent of the body-weight to 0.64 per cent at six weeks, 0.82 per cent at eight weeks, and

TABLE 11

The spinal cord; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Donaldson '08, table 1)	47	25.0*	0.180	0.72
Controls at 3 weeks	11	24.5	0.179 (0.152–0.200)	0.74 (0.56–0.89)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	6	22.0	0.207 (0.149–0.235)	0.96 (0.63–1.25)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.192 (0.188–0.195)	1.08 (1.06–1.09)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	0.243 (0.201–0.314)	1.02 (0.89–1.17)
Normal at 6 weeks (Donaldson '08)	42	45.0*	0.254	0.57
Controls at 6 weeks	1	42.4	0.286	0.67
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	0.399 (0.378–0.420)	0.85 (0.83–0.86)
Normal at 10 weeks (Donaldson '08)	32	75.0*	0.333	0.44
Controls at 10 weeks†	6	134.0	0.422 (0.341–0.464)	0.32 (0.27–0.39)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	0.520 (0.500–0.550)	0.67 (0.64–0.69)
Controls at 32 and 35 weeks	6	189.5	0.603 (0.491–0.667)	0.33 (0.24–0.44)

* Gross body-weight.

† Body-weight of controls at 10 weeks too high for comparison.

TABLE 12

The eyeballs; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	24	21.2	0.105 (0.073–0.125)	0.52 (0.31–0.73)
Controls at 3 weeks	10	24.6	0.120 (0.110–0.133)	0.50 (0.34–0.69)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	6	22.1	0.142 (0.138–0.149)	0.64 (0.60–0.72)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.146 (0.145–0.146)	0.82 (0.81–0.82)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	0.179 (0.130–0.196)	0.76 (0.57–1.00)
Normal at 6 weeks (Jackson '13)	42	50.0	0.153 (0.125–0.175)	0.32 (0.18–0.40)
Control at 6 weeks	1	42.4	0.152	0.36
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	0.240 (0.230–0.250)	0.51 (0.50–0.52)
Normal at 10 weeks (Jackson '13)		75.0	0.173	0.23
Controls at 10 weeks*	6	134.0	0.209 (0.195–0.228)	0.16 (0.13–0.21)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	0.275 (0.249–0.323)	0.35 (0.34–0.38)
Controls at 32 and 35 weeks	5	184.4	0.260 (0.235–0.296)	0.15 (0.12–0.17)

* Body-weight of controls at 10 weeks too high for comparison.

0.76 per cent at ten weeks. In terms of absolute weight, the eyeballs have apparently increased from an average of 0.120 grams at three weeks to about 0.179 grams (no correction made for the slight difference in body-weight) at ten weeks, an increase of nearly 50 per cent! In the normal, full-fed rat at ten weeks (average body-weight 112 grams) the eyeballs have reached a weight of only about 0.201 grams (Jackson '13). At this rate, the weight of the eyeballs at a normal body-weight of 75 grams (the body-weight indicated in table 12 as 'normal at 10 weeks.' to correspond to the body-weight of the animals held at constant body-weight from the age of ten to thirty-five weeks) would be only about 0.173 grams, or slightly less than that actually reached in the series held at constant body-weight of 24 grams from three weeks to ten weeks of age. The growth of the eyeballs in rats held constant from the age of six to thirty-two weeks, and from ten to thirty-five weeks, is equally striking.

No data upon the growth of the eyeballs under these conditions have been found in the literature. I have shown elsewhere (Jackson '15 a, '15 c), however, that the eyeballs lose but very little if any during inanition in the adult albino rat.

In connection with the astonishing growth capacity of the eyeballs in young animals at constant body-weight, the possibility that the growth of the eyeballs is somewhat independent of that in the body as a whole may be considered, which I have already pointed out (Jackson '13, p. 24). When the large water-content of the eyeballs is considered (85.6 per cent in the rat at twenty days, according to Lowrey '13), it is, after all, not difficult to comprehend the possibility of its continued growth, largely by water-absorption, when growth in the body as a whole is at a standstill.

THYROID GLAND

In young albino rats held at constant body-weight from the age of three weeks to six weeks, eight weeks and ten weeks, there is usually a well-marked loss of weight in the thyroid gland (table 13). In the largest group, three to ten weeks, the thyroid has apparently decreased on the average from about 0.033 per cent

TABLE 13

The thyroid gland; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	26	18.7	0.0060 (0.0034–0.0104	0.030 (0.018–0.041)
Controls at 3 weeks	11	24.6	0.0078 (0.0064–0.0094	0.033 (0.022–0.042)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	3	23.1	0.0053 (0.0052–0.0056	0.023 (0.022–0.024)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.0058 (0.0047–0.0068	0.032 (0.026–0.038)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	18	24.1	0.0059 (0.0034–0.0087	0.025 (0.016–0.036)
Normal at 10 weeks (Jackson '13)	24	110.0	0.0165 (0.0100–0.030)	0.015 (0.008–0.022)

of the body to 0.025 per cent. Or, in terms of absolute weight, it has decreased from 0.0078 to 0.0059 grams, a decrease of 24 per cent. (A slight correction should be made on account of difference in body-weight.) No observations were made upon the thyroid gland in the experiments beginning at later ages.

During acute inanition in adult rats, the thyroid gland apparently loses little or no weight; while in chronic inanition with an average loss in body-weight of about 36 per cent, the thyroid gland loses only about 22 per cent in weight (Jackson '15). There is some uncertainty as to the exact figures, however, on account of variability and difficulty in dissecting out the thyroid gland in an accurate manner. The same, of course, holds true for the present series.

THYMUS

The normal thymus (table 14) at three weeks forms 0.37 per cent of the net body-weight. This decreases, in rats held at constant body-weight, to 0.075 per cent at six weeks of age, to 0.030 (exceptional?) at eight weeks, and to 0.040 per cent at ten weeks. In terms of absolute weight, the thymus has decreased from 0.091 grams at three weeks to 0.017 grams (loss of 81 per cent) at six weeks, and to 0.0094 grams (loss of 90 per cent) at 10 weeks. No correction for the slight difference in body-weight has been made in these estimates.

Normally at ten weeks of age the weight of the thymus should have increased to about 0.24 grams (0.30 grams in the controls).

A maximum absolute weight of about 0.29 grams is reached by the average normal thymus about the age of 85 days (Hatai '14). At one year, it has undergone a complete age-involution, and forms only 0.02 per cent of the body-weight (Jackson '13).

That the weight and structure of the thymus are markedly affected by various adverse conditions has long been known, and the process of involution has recently been thoroughly investigated by Hammar and his pupils. Jonson ('09) experimented with young rabbits subjected to acute and chronic inanition. In the latter case, the diet was restricted so as to maintain the young rabbits at constant body-weight (similar to the present experiment with rats). Under these conditions, Jonson found the weight-curve of the thymus similar to that of the body-fat, although during *acute* inanition the fat decreases somewhat more rapidly. In young rabbits held at constant body-weight the thymus in four weeks is reduced to about one-thirtieth of its initial weight. The cortex suffers the greatest loss, being reduced to one-twelfth of its initial weight within two weeks of chronic inanition at constant body-weight. It would therefore appear that the process of involution is much more rapid and complete in young rabbits than in young rats at the ages included in the present investigation. In both cases, however, the weight of the thymus in hunger involution decreases most rapidly in the earlier weeks of the experiment.

HEART

The heart (table 15) in the albino rats held at constant body-weight from the age of three weeks appears to have remained practically constant at about 0.70 per cent of the net body-weight up to the age of ten weeks. In absolute weight, the heart would apparently decrease from 0.167 grams (0.170 grams, less correction for difference in body-weight) to 0.166 grams, a decrease of about 0.6 per cent, which is probably within the limits of experimental error. The very slight decrease at six weeks and increase at eight weeks are also probably not significant. Similarly in the rats held at constant body-weight from six to thirty-two weeks and from ten to thirty-five weeks the heart has apparently retained almost exactly its initial relative weight. The absolute weight therefore apparently remains unchanged (the differences in the table being due to different initial body-weights).

During inanition in adult rats (Jackson '15 a, '15 c) the heart likewise maintains its relative weight, losing in absolute weight nearly in proportion to the entire body (slightly more in chronic than in acute inanition).

TABLE 14

The thymus; average absolute weight, average percentage weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGTH (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	49	18.7	0.071 (0.034 –0.135)	0.37 (0.23 –0.55)
Controls at 3 weeks	11	24.5	0.091 (0.042 –0.123)	0.37 (0.22 –0.51)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	4	22.7	0.017 (0.011 –0.022)	0.075 (0.046–0.0101
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.0054 (0.0037–0.0071)	0.030 (0.021–0.040)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	0.0094 (0.0054–0.0170)	0.040 (0.019–0.062)
Normal at 6 weeks (Jackson '13)	42	50.0	0.108 (0.052 –0.284)	0.21 (0.14 –0.36)
Normal at 10 weeks (Jackson '13)	42	107.2	0.24 (0.12 –0.44)	0.23 (0.13 –0.35)
Controls at 10 weeks	6	134.0	0.30 (0.25 –0.34)	0.23 (0.16 –0.29)

TABLE 15

The heart; average absolute weight; average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	49	18.7	0.135 (0.082–0.250)	0.72 (0.56–0.93)
Controls at 3 weeks	11	24.5	0.170 (0.130–0.245)	0.70 (0.55–0.84)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	0.151 (0.123–0.187)	0.68 (0.63–0.85)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.129 (0.128–0.130)	0.72 (0.72–0.72)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	0.166 (0.124–0.213)	0.70 (0.55–0.83)
Normal at 6 weeks (Jackson '13)	42	50.0	0.277 (0.183–0.535)	0.55 (0.44–0.68)
Controls at 6 weeks	1	42.4	0.237	0.56
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	0.271 (0.255–0.288)	0.57 (0.57–0.58)
Normal at 10 weeks (Jackson '13)		75.0	0.375	0.50
Controls at 10 weeks*	6	134.0	0.562 (0.489–0.687)	0.44 (0.40–0.48)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	0.389 (0.348–0.450)	0.50 (0.47–0.52)
Controls at 32 and 35 weeks	6	189.5	0.873 (0.688–1.29)	0.46 (0.37–0.56)

* Body-weight of controls at 10 weeks too high for comparison.

LUNGS

The lungs (table 16) in rats held at constant body-weight from the age of three weeks to the ages of six weeks and ten weeks appear to lose in weight. (The data at eight weeks are abnormally high, as noted in the table). Thus between the ages of three weeks and ten weeks, the lungs apparently decrease from 1.04 per cent to 0.91 per cent of the body-weight; or in absolute weight from 0.250 to 0.218 grams, a loss of about 15 per cent. (A slight correction has been made on account of the difference in body-weight). In the few observations upon rats for longer periods beginning at the later ages of six and ten weeks, there appears to be no material change in the weights of the lungs during the experimental periods.

The lung infections frequently found in older and adult rats, rarely occur before the age of ten weeks, and thus do not affect the present series. As Hatai has already noted, rats during chronic inanition appear to be unusually free from lung infection.

During inanition in adult rats, the lungs lose weight in about the same proportion as the whole body, thus nearly maintaining their relative (percentage) weight. The loss is slightly greater, however, during chronic inanition (Jackson '15 c).

TABLE 16

The lungs; average absolute weight; average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	49	18.7	0.216 (0.151–0.354)	1.17 (0.86–1.46)
Controls at 3 weeks	11	24.5	0.253 (0.201–0.290)	1.04 (0.89–1.12)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	3	20.9	0.192 (0.190–0.197)	0.92 (0.88–0.93)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.280* (0.241–0.319)	1.56* (1.36–1.77)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	0.218 (0.170–0.282)	0.91 (0.78–1.32)
Normal at 6 weeks (Jackson '13)	39	50.0	0.333 (0.244–0.547)	0.68 (0.58–0.94)
Control at 6 weeks	1	42.4	0.264	0.62
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	0.319 (0.309–0.329)	0.68 (0.65–0.70)
Normal at 10 weeks (Jackson '13)		75.0	0.49	0.65
Controls at 10 weeks	6	134.0	0.74 (0.610–0.94)	0.56 (0.45–0.74)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	0.49 (0.390–0.60)	0.63 (0.52–0.81)

* Lungs in one case abnormally heavy on account of post mortem congestion.

LIVER

The liver (table 17) in the albino rats held at constant body-weight from the age of three weeks to six, eight and ten weeks shows an apparent increase. This increase appears greatest in the shortest series, three to six weeks, increasing from 4.95 per cent of the body-weight (in controls) to 5.89 per cent. Between three and ten weeks the corresponding increase is from an absolute weight of 1.16 grams (1.20 grams, less correction for difference in body-weight; or 4.95 per cent of the body-weight) to 1.28 grams (5.25 per cent of the body-weight), or an increase of about 10.3 per cent in absolute weight.

In the later and longer periods, however, there appears to be a decided decrease in the weight of the liver (six to thirty-two weeks and ten to thirty-five weeks series). This may be due to the fact that the latter experiments extended over a longer period of time, or it may be because they were begun at a later age. We may therefore conclude that in young albino rats held at constant body-weight there is, beginning at three weeks, an increase in the weight of the liver (apparently greater at six than at ten weeks of age); while in rats beginning the experiment later, at six and ten weeks of age (and extending over a longer period)

TABLE 17

The liver; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	49	18.7	0.87 (0.42–2.08)	4.50 (3.21–5.82)
Controls at 3 weeks	11	24.5	1.20 (1.06–1.38)	4.95 (4.03–6.00)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	1.30 (0.89–1.63)	5.89 (4.32 –7.39)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.92 (0.78–1.06)	5.12 (4.36–5.88)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	1.28 (0.80–1.82)	5.25 (3.63–7.00)
Normal at 6 weeks (Jackson '13)	42	50.0	3.19 (2.18–4.76)	6.48 (5.39–8.46)
Control at 6 weeks	1	42.4	2.18	5.15
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	2.33 (2.21–2.45)	4.44
Normal at 10 weeks (Jackson '13)		75.0	4.50	6.00
Controls at 10 weeks	6	134.0	6.72 (4.91–8.66)	4.99 (4.28–5.72)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	3.11 (2.30–3.51)	4.25 (3.91–4.74)
Controls at 32 and 35 weeks	6	189.5	7.27 (5.84–9.86)	3.84 (3.47–4.14)

there is a decided decrease in the weight of the liver. Thus the liver would appear under these conditions to have a tendency to follow the normal growth-impulse, which increases to a maximum at about the age of six weeks, and decreases thereafter (Jackson '13, p. 31).

During inanition in adult rats, the liver loses in weight relatively more than the whole body, and to a greater extent in acute than in chronic inanition (Jackson '15 c). As the liver is normally subject to great variation in weight, however, (Jackson '13) great caution must be observed in drawing final conclusions. Hatai ('13) found the weight of the normal liver distinctly higher than that in my series, and if his data instead of my controls were taken as a basis for the initial weights, the estimated losses in my experiments would be considerably greater, even the younger rats showing a loss instead of a gain in liver-weight.

SPLEEN

Taking the controls at three weeks as a basis for comparison, it appears that in rats held at constant body-weight from the age of three weeks to six weeks the weight of the spleen (table 18) remains practically unchanged (the average at six

TABLE 18

The spleen; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	49	18.7	0.055 (0.019–0.145)	0.28 (0.15–0.42)
Controls at 3 weeks	11	24.5	0.091 (0.051–0.130)	0.37 (0.27–0.48)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	0.087 (0.040–0.172)	0.38 (0.18–0.72)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.043 (0 036–0.049)	0.24 (0.20–0.27)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	0 053 (0.033–0.076)	0.22 (0.16–0.33)
Normal at 6 weeks (Jackson '13)	42	50.0	0.135 (0.086–0.204)	0.28 (0.19–0.47)
Control at 6 weeks	1	42.4	0.107	0.25
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	0.139	0.29 (0.28–0.30)
Normal at 10 weeks (Jackson '13)		75.0	0 225	0.30
Controls at 10 weeks	6	134.0	0.350 (0.300–0.420)	0.27 (0.21–0.36)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	0.230 (0.230–0.240)	0.30 (0.27–0.33)
Controls at 32 and 35 weeks	6	189.5	0.620 (0.480–0.750)	0.33 (0.29–0.39)

weeks being probably too high, on account of the inclusion of a litter with abnormally large spleens); while in the rats at eight and ten weeks there is a considerable decrease. In the case of the three to ten weeks series, the decrease would be from 0.091 gram (0.37 per cent of the net body-weight) to 0.053 gram (0.22 per cent of the body-weight), or a decrease of nearly 42 per cent in absolute weight. (No correction has been made for the difference in body-weight, which is 0.5 gram lower at ten weeks). If the normal relative weight of the spleen at three weeks (0.28 per cent, Jackson '13) be taken as a basis of estimate, however, the loss would appear considerably less.

In the rats held at constant body-weight at later and longer periods (six to thirty-two weeks and ten to thirty-five weeks) there appears to be no appreciable change in the average weight of the spleen. It may be concluded therefore that in young rats held at constant body-weight beginning at the age of three weeks there is a marked tendency to a reduction in the weight of the spleen; while at later (and longer) periods the spleen appears to undergo no material change in weight. It must be remembered, however, that the spleen is normally one of the most variable organs in the body (Jackson '13), and final conclusions should be correspondingly guarded.

In adult albino rats during chronic inanition the average loss in weight is nearly proportional to that of the entire body, while in acute inanition the loss appears very much greater (Jackson '15 a, '15 c).

STOMACH AND INTESTINES

The stomach and intestines, including mesentery and pancreas, are considered both with contents (table 19 a) and empty (table 19 b). Considering first the empty canal, it appears in rats held at constant body-weight from the age of three weeks to increase from about 4.8 per cent of the body-weight to 8.0 per cent at six and eight weeks, decreasing to 6.0 per cent at ten weeks. In absolute weight the increase would be from about 1.13 grams (1.20 grams less correction on account of difference in body-weight) at three weeks to 1.45 grams at ten weeks, an increase of about 28 per cent. Between six and thirty-two weeks there is apparently but little change; while from ten to thirty-five weeks there appears to be a decrease in the weight of the alimentary canal. The number of observations, however, is too small for final conclusion.

The most remarkable increase occurs between three and six weeks of age, from an absolute weight of 1.20 to 1.78 grams, an apparent increase of about 48 per cent in absolute weight! (The increase would be even greater if allowance were made for difference in body-weight). Thus, as in the case of the liver and

TABLE 19

The stomach and intestines; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
a. Including contents				
Normal at 3 weeks (Jackson '13)	49	18.7	1.78 (0.74–3.08)	9.3 (5.5–15.5)
Controls at 3 weeks	11	24.5	2.51 (1.96–3.23)	10.4 (7.8–15.4)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	3.10 (1.67–5.07)	13.9 (7.4–21.3)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	3.64 (2.58–4.71)	20.2 (4.5–26.0)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	3.29 (2.26–6.83)	13.5 (10.3–20.2)
Normal at 6 weeks (Jackson '13)	42	50.0	8.05 (4.48–13.40)	15.9 (10.6–23.4)
Controls at 6 weeks	1	42.4	5.13	12.1
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	5.98 (4.71–7.24)	12.5 (10.8–14.2)
Normal at 10 weeks (Jackson '13)		75.0	9.00	12.0
Controls at 10 weeks*	6	134.0	11.32 (8.82–14.49)	8.3 (7.3–11.2)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	7.48 (5.90–8.81)	9.6 (8.1–10.4)
Controls at 32 and 35 weeks	6	189.5	11.60 (7.51–14.04)	7.4 (5.4–12.2)
b. Empty				
Normal at 3 weeks (Jackson '13)	16	20.0	0.90 (0.37–1.61)	4.5 (2.9–6.1)
Controls at 3 weeks	10	25.1	1.20 (0.74–1.69)	4.8 (3.1–6.7)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	1.78 (1.41–2.51)	8.0 (6.3–11.0)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	1.44 (1.38–1.51)	8.0 (7.8–8.3)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	1.45 (0.96–2.29)	6.0 (4.5–8.6)
Normal at 6 weeks (Jackson '13)		50.0	4.00	8.0
Controls at 6 weeks	1	42.4	3.00	7.1
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	3.22 (2.85–3.59)	6.8 (6.5–7.0)
Normal at 10 weeks (Jackson '13)		75.0	5.30	7.0
Controls at 10 weeks*	5	127.9	5.26	4.3 (3.9–4.9)
Body-weight constant 25 weeks (age of 10 to 25 weeks)	3	77.8	3.97 (3.48–4.35)	5.4 (4.7–5.9)
Controls at 32 and 35 weeks	6	189.5	9.16 (7.57–10.64)	4.9 (4.1–5.3)

* Body-weight of controls at 10 weeks too great for comparison with those held constant 10–35 weeks.

following the normal growth tendency, there appears to be in these retarded rats an early increase in the weight of the alimentary canal, reaching a maximum at about the age of six weeks, after which there is a decline in weight. It may be that the early increase in these organs is diminished later on account of the exhaustion of the stored food-supplies available elsewhere in the body at the beginning of the experiment. Individual organs and tissues differ greatly from each other in their relative susceptibility to attack and absorption at different times during the course of inanition.

The behavior of the stomach and intestines weighed with contents (table 19 a) is very similar to that of the empty canal. Contrary to what might be expected, during constant body-weight, with a restricted food-supply (water *ad libitum*), the canal does not decrease in contents. On the contrary there is an *increase* in contents (watery or mucous in character) which may even exceed in relative weight the normal contents in full-fed aminals. The maximum occurred in animals held at constant body-weight from the age of three to eight weeks, when the canal with contents formed 20.2 per cent of the body-weight! In experiments beginning at later ages and extending over longer periods (six to thirty-two weeks and ten to thirty-five weeks), the canal with contents appears to remain more nearly uniform in weight, with some tendency to decrease.

During both acute and chronic inanition in adult albino rats, there is a very marked decrease in the weight of the stomach and intestines, both with and without contents (Jackson '15 a, '15 c).

SUPRARENAL GLANDS

The suprarenal glands (table 20) from the age of about six weeks must be considered separately in the sexes, on account of a distinct sexual difference in their weight, as discovered independently by Hatai ('13) and myself (Jackson '13). In the earlier ages, however, there is no apparent sexual difference, hence the sexes are combined.

TABLE 20

The suprarenal glands; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks* (Jackson '13)	49	18.7	0.0072 (0.0040–0.0140)	0.0400 (0.023–0.074)
Controls at 3 weeks*	11	24.5	0.0088 (0.0060–0.0108)	0.0370 (0.025–0.055)
Body-weight constant 3 weeks* (age of 3 to 6 weeks)	7	22.1	0.0089 (0.0070–0.0110)	0.0400 (0.034–0.050)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	1m	18.1	0.0109	0.0610
	1f	17.8	0.0088	0.0490
Body-weight constant 7 weeks (age of 3 to 10 weeks)	7m	25.2	0.0101 (0.0072–0.0126)	0.0420 (0.033–0.052)
	12f	23.3	0.0117 (0.0090–0.0147)	0.0510 (0.040–0.061)
Normal at 6 weeks* (Jackson '13)	42	50.0	0.0128 (0.0070–0.0190)	0.0260 (0.015–0.039)
Control at 6 weeks	1f	42.4	0.0090	0.0210
Body-weight constant 26 weeks (age of 6 to 32 weeks)	1m	43.7	0.0105	0.0240
	1f	50.5	0.0090	0.0180
Normal at 10 weeks (Jackson '13)	20m	117.0	0.0220 (0.0150–0.0336)	0.0185 (0.011–0.025)
	23f	99.0	0.0253 (0.0180–0.0329)	0.0257 (0.017–0.033)
Controls at 10 weeks	3m	154.7	0.0295 (0.0270–0.0326)	0.0190 (0.017–0.023)
	3f	114.0	0.0318 (0.0290–0.0346)	0.0280 (0.027–0.031)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	2m	79.6	0.0149 (0.0147–0.0150)	0.0190 (0.017–0.020)
	1f	74.2	0.0145	0.020
Controls at 32 and 35 weeks	3m	218.7	0.0270 (0.0180–0.0310)	0.0100 (0.009–0.013)
	3f	160.3	0.0320 (0.0280–0.0360)	0.0200 (0.017–0.024)

* No sexual difference apparent.

TABLE 21

The kidneys; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	49	18.7	0.271 (0.169–0.531)	1.44 (1.19–1.87)
Controls at 3 weeks	11	24.5	0.393 (0.314–0.487)	1.62 (1.33–2.16)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	0.396 (0.328–0.487)	1.80 (1.46–2.05)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.339 (0.331–0.347)	1.89 (1.86–1.92)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	0.404 (0.325–0.515)	1.69 (1.44–1.97)
Normal at 6 weeks (Jackson '13)	42	50.0	0.616 (0.500–0.943)	1.25 (1.00–1.55)
Controls at 6 weeks	1	42.4	0.570	1.35
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	0.613 (0.581–0.645)	1.30 (1.28–1.33)
Normal at 10 weeks (Jackson '13)		75.0	0.750	1.00
Controls at 10 weeks*	6	134.0	1.200 (0.953–1.481)	0.91 (0.81–0.99)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	0.753 (0.728–0.784)	0.97 (0.92–1.02)
Controls at 32 and 35 weeks	6	189.5	1.539 (1.275–1.909)	0.81 (0.71–0.86)

* Controls too heavy for comparison.

In the rats held at constant body-weight from the age of three weeks the suprarenal glands appear to increase slightly in weight at six and eight weeks; and especially at ten weeks, where the characteristic sexual difference has distinctly appeared. In absolute weight, the suprarenal glands have increased from 0.0090 gram (0.0088 gram, plus correction for difference in body-weight; or 0.037 per cent of the body-weight) at three weeks to 0.0101 gram (0.042 per cent of the body-weight) in the male at ten weeks, an increase of about 12 per cent in absolute weight. In the female, the corresponding increase is from 0.0084 gram (0.0088 gram, less correction for difference in body-weight) (or 0.037 per cent of the body-weight) to 0.0117 gram (0.051 per cent of the body-weight), an increase of 39 per cent in absolute weight. Normally the suprarenals during this period are decreasing in relative (percentage) weight. In the experiments at later and longer periods (six to thirty-two weeks and ten to thirty-five weeks), the changes are apparently not great, but a larger number of observations is necessary before definite conclusions can be reached.

In adult rats during inanition there is little or no loss in the absolute weight of the suprarenal glands, which therefore increase markedly in relative (percentage) weight (Jackson '15 a, '15 c).

KIDNEYS

The kidneys (table 21) in rats held at constant body-weight from the age of three weeks show a tendency to increase which is more marked at six and eight than at ten weeks. Between three and ten weeks of age the increase is from an average of 0.388 gram (0.393 gram, less correction on account of difference in body-weight; or 1.62 per cent of the body-weight) to 0.404 gram (1.69 per cent of the body-weight), an increase of only about 4.1 per cent in absolute weight. At later and longer periods (six to thirty-two weeks and ten to thirty-five weeks) there is apparently but little change in the weight of the kidneys. The slight differences shown in the table are probably not significant. On the whole, it appears that in young rats held at constant body-weight there is a slight tendency to increase in the weight of the kidneys, in the earlier weeks, but little or no apparent difference later.

In adult rats during acute and chronic inanition, the kidneys lose in weight relatively slightly less than the body as a whole, thereby gaining slightly in relative (percentage) weight (Jackson '15 c).

TESTES AND EPIDIDYMI

The weights of testes and epididymi unfortunately were not separated in some cases, which (together with their variability and the small number of observations) makes conclusions somewhat difficult. For the testis (table 22 a), the clearest case is in rats held constant from three to ten weeks of age, in which

TABLE 22

The testes and epididymi; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
a. Testes				
Normal at 3 weeks (Hatai '13)		20.0*	0.090	0.45
Normal at 3 weeks (incl. epididymi) (Jackson '13)	24	21.2	0.134† (0.076-0.224)	0.63* (0.53-0.78)
Controls at 3 weeks	6	25.0	0.144 (0.114-0.200)	0.57 (0.49-0.62)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	2	22.1	0.133† (0.129-0.137)	0.66* (0.65-0.67)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	1	18.1	0.176†	0.98*
Body-weight constant 7 weeks (age of 3 to 10 weeks)	7	25.2	0.193 (0.106-0.331)	0.74 (0.49-1.05)
Normal at 6 weeks (Hatai '13)		50.0*	0.402	0.80
Normal at 6 weeks (Jackson '13)	20	49.0	0.592† (0.368-0.958)	1.19* (0.88-1.72)
Body-weight constant 26 weeks (age of 6 to 32 weeks)	1	43.7	0.370†	0.84*
Normal at 10 weeks (Hatai '13)		70.0*	0.774	1.11
Normal at 10 weeks (Jackson '13)	20	117.0	1.747† (0.678-2.62)	1.51* (0.63-2.41)
Controls at 10 weeks	3	154.7	1.850 (1.608-2.10)	1.21* (1.06-1.49)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	2	79.6	1.294† (1.401-1.188)	1.62* (1.61-1.64)
Controls at 32 and 35 weeks	3	218.7	1.844† (1.766-1.932)	0.84* (0.74-0.90)
b. Epididymi				
Normal at 3 weeks (Jackson '13) (estimated)		20.0	0.200 (?)	0.10 (?)
Controls at 3 weeks	6	25.0	0.0209 (0.0092-0.0246	0.084 (0.039-0.123)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	1	23.6	0.0144	0.061
Body-weight constant 7 weeks (age of 3 to 10 weeks)	7	25.2	0.0209 (0.0084-0.0360)	0.080 (0.040-0.115)
Controls at 10 weeks	3	154.7	0.4700 (0.4480-0.4920)	0.310 (0.290-0.330)

* Gross body-weight.

† Including epididymi.

there is an increase from 0.144 gram (0.57 per cent of the body-weight) to 0.193 gram (0.74 per cent of the body), an apparent increase of 34 per cent in the absolute weight. (The average net body-weight was about the same in both cases, 25.0 grams in the controls, and 25.2 grams at ten weeks).

For the epididymi (table 22 b), the conclusions are even more, uncertain; but there appears to be a slight loss in the relative weight of the epididymi in rats held at constant body-weight from the age of three to six and ten weeks.

For testis and epididymis combined (table 22 a) there appears to be an increase in rats held at constant body-weight, excepting the period from six to thirty-two weeks.

While no final conclusions can be drawn, the evidence indicates that in young rats held at constant body-weight there is an increase in the weight of the testis, but not in the epididymis.

During inanition in adult rats, the testes and epididymi apparently lose weight in about the same proportion as the entire body (Jackson '15 c).

OVARIES

In rats held at constant body-weight from three weeks to ten weeks of age, there would appear to be a decrease in the weight of the ovaries (table 23) from 0.0066 gram (0.0068 gram, less correction for difference in body-weight; or 0.027 per cent of the

TABLE 23

The ovaries; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13)	24	16.2	0.0036 (0.0015–0.0067)	0.0220 (0.011–0.039)
Controls at 3 weeks	5	24.5	0.0068 (0.0029–0.0104)	0.0270 (0.012–0.034)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	3	22.4	0.0067 (0.0048–0.0084)	0.0300 (0.020–0.038)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	12	23.3	0.0048 (0.0030–0.0064)	0.0210 (0.012–0.028)
Normal at 6 weeks (Jackson '13)	20	54.9	0.0106 (0.0050–0.028)	0.0216 (0.012–0.035)
Normal at 10 weeks (Jackson '13)	23	98.8	0.0350 (0.0100–0.070)	0.0340 (0.013–0.055)
Controls at 10 weeks	3	114.0	0.0337 (0.0297–0.038)	0.0290 (0.027–0.033)

body-weight) to 0.0048 gram (0.021 per cent of the body-weight). This would indicate a decrease of about 27 per cent in absolute weight. The ovary, however, is an organ which (like the thyroid gland) is quite variable and somewhat difficult to dissect out with accuracy, so a much larger number of observations would be required for a final conclusion.

HYPOPHYSIS

In the hypophysis (table 24) there is normally a sexual difference in weight, observable in rats above 50 grams in body-weight (Hatai '13). As in the case of the suprarenal glands, the hypophysis normally becomes relatively heavier in the female.

In the rats held at constant body-weight from the age of three weeks to six weeks, there is what appears to be a sexual difference, the one male with a hypophysis of 0.0016 gram (0.0068 per cent of the body-weight), while the two female hypophyses each weigh 0.0018 gram (0.0079 per cent of the body-weight). These are probably mere accidental variations, however, as in the three to ten weeks series the difference in the relative weight is insignificant (0.0084 per cent of the body-weight in the males, and 0.0086 per cent in the females). In any event, however,

TABLE 24

The hypophysis; average absolute weight, average percentage of net body-weight and range indicated

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE) PER CENT
Normal at 3 weeks* (Hatai '13)		25.0†	0.00175	0.0070
Controls at 3 weeks*	9	25.5	0.00178 (0.0012–0.0022)	0.0070 (0.0052–0.0084)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	1m.	23.6	0.0016	0.0068
	2f.	22.9	0.0018	0.0079 (0.0076–0.0082)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	7m.	25.2	0.0021 (0.0016–0.0024)	0.0084 (0.0057–0.0110)
	11f.	23.6	0.0020 (0.0010–0.0033)	0.0086 (0.0043–0.0135)
Normal at 10 weeks (Hatai '13)	m.	130.0	0.0054	0.0042
	f.	130.0	0.0084	0.0065
Controls at 10 weeks	3m.	154.7	0.0062 (0.0059–0.0067)	0.0040 (0.0038–0.0042)
	3f.	114.0	0.0054 (0.0046–0.0067)	0.0048 (0.0040–0.0062)

* No sexual difference apparent.

† Gross body-weight.

there appears to be a distinct tendency to an increase in the weight of the hypophysis in rats held at constant weight from three to ten weeks of age. In absolute weight, this corresponds in the male to an increase from 0.00178 gram to 0.0021 gram, an increase of about 18 per cent. In the female, the corresponding increase is from 0.00168 gram (0.00178 gram, less correction for difference in body-weight) to 0.0020 gram, an increase of 19 per cent.

As already shown, the suprarenal glands differ from the hypophysis in that they undergo a marked sexual differentation in weight during their growth while the body-weight is held constant. The impulse to sexual differentiation in these glands therefore appears stronger in the suprarenals than in the hypophysis. Final conclusions should be guarded, however, until a larger number of observations is available.

In the adult rat during inanition the weight of the hypophysis decreases in nearly the same proportion as the body-weight (Jackson '15 c).

DISCUSSION

With reference to their growth tendency in young rats held at constant body-weight, the organs may be divided into three classes: (1) those in which the growth tendency is so strong that they continue to increase, even when the body-weight is held constant; (2) those which approximately hold their weight constant under these conditions; and (3) those which are unable to maintain themselves and lose in weight.

According to this scheme, the organs in rats held at constant body-weight from the age of three to that of ten weeks are grouped in table 25. No grouping of this sort can be entirely satisfactory, because in some cases organs are intermediate in position, and especially because (as has been shown) in many cases the weight of an organ will vary according to the age at which the experiment was begun and the length of the period. For example, the liver, which in the three-to-ten weeks experiment shows but a slight gain, shows a larger gain at earlier times, and a loss at later and longer periods.

On the whole, however, the grouping suffices to give a good view of the results. Group I shows the organs with marked growth tendency to be the skeleton, eyeballs, spinal cord, alimentary canal, testes, hypophysis and suprarenal glands. Group II, which approximately maintains constant weight, includes the musculature, brain, heart, kidneys and liver. Group III, those which fail to maintain their weight when the body-weight is held constant, includes the integument, lungs, thyroid gland, ovaries, spleen and thymus.

In the same table 25 for convenience of comparison are also grouped the various organs of the adult rat according to their relative loss in weight during chronic inanition (Jackson '15 c).

TABLE 25

Comparison of growth tendency in young rats held at constant body-weight from age of three to ten weeks with tendency to maintenance in adult rats during chronic inanition. The figures indicate the apparent average percentage gain or loss in absolute weight during the period of experiment

	GROUP I		GROUP II		GROUP III	
	Marked tendency to growth in young held at constant body-weight. Strong tendency to maintenance in adult chronic inanition		Weight nearly constant in young held at constant body-weight. Relative loss similar to that of entire body during adult chronic inanition		Marked loss in weight in young held at constant body-weight. Relative loss greater than that of entire body during adult chronic inanition	
		per cent of change		*per cent of change*		*per cent of change*
Young at constant body weight....	*eyeballs	+50.0	liver	+10.3	lungs	−15.0
	*spinal cord	+36.0	*kidneys	+4.1	thyroid gland	−24.0
	testes	+34.0	*musculature	+3.0	ovaries	−27.0
	*skeleton	+28.0	brain	−0.5	integument	−36.0
	alimentary canal	+28.0	*heart	−0.6	spleen	−42.0
	*suprarenal glands	M. +12.0 F. +39.0			thymus	−90.0
	hypophysis	M. +18.0 F. +19.0				
Adults during chronic inanition (loss of body-weight 36 per cent)	*skeleton	+1.8	hypophysis	−25.3	liver	−43.0
	*spinal cord	−4.0	*kidneys	−26.8	alimentary canal	−57.0
	*eyeballs	−5.8	spleen	−29.0		
	brain	−6.6	*heart	−32.8		
	*suprarenal glands	−8.9	integument	−38.5		
	thyroid gland	−21.8	lungs	−40.0		
	thymus	(?)	testes	−40.3		
			*musculature	−40.8		

* Indicates correspondence between the groupings in young and adult series.

This enables us to answer the question as to whether the organs in young animals held at constant body-weight (and thus subjected to a chronic inanition) behave in a manner similar to adult rats during chronic inanition. In many cases, the grouping shows an agreement. The skeleton, eyeballs, spinal cord and suprarenal glands have a strong growth tendency in young held at constant body-weight, and also a strong tendency to maintain their original weight in adults subjected to chronic inanition. The musculature, heart and kidneys approximately maintain their weight in young held constant, and maintain their *relative* weight in adults during chronic inanition. In the majority of cases, however, the behavior of organs in the young differs materially from that in the adult. Thus the alimentary canal has a marked growth in the young at constant weight, yet it loses heavily during adult inanition. The converse is apparently true of the thyroid gland. To a greater or less degree, this inconsistency is seen in the case of most of the organs, as is evident from table 25.

This inconsistency is perhaps to be explained in the following manner. In the adult during inanition the various organs lose weight relatively in inverse ratio to the ability of their cells to extract nutrition from the diminishing quantity available in the surrounding medium and to maintain equilibrium under these adverse conditions. In the young animal held at constant body-weight, the conditions differ in that the corresponding cells have the capacity not only to *maintain* themselves, but to *grow*. The growth capacity, as is well known, is different from and to some extent independent of the maintenance capacity. The adult has lost the capacity to grow, whereas the young animal has both the powers of growth and maintenance. Hence their organs behave differently during inanition. The alimentary canal, for example, apparently has a strong growth tendency, but a weak power of maintenance.

It is further evident, however, that even the growth capacities of the various tissues and organs differ relatively from each other under different planes of nutrition. Thus under normal conditions of growth in the rat between three and ten weeks of age the *muscular* system shows the strongest growth capacity, and increases with greater relative rapidity than any other system (Jackson and Lowrey). The skeleton is of course growing steadily, but at a much slower rate, so that it is decreasing in relative (percentage) weight. Under the adverse nutritive conditions in young animals when the body-weight is held constant, however, the musculature is barely able to maintain its weight, while the skeleton is able to absorb more than its share of the available nutrition, and to grow steadily (though at a retarded rate). There are similar differences among many of the individual organs, though in some cases (e.g., liver, alimentary canal) there is a certain degree of parallelism between the normal growth tendency and the behavior when the body-weight is held constant at corresponding periods.

That also the power of maintenance may vary in organs according to the nutritional conditions is shown by the characteristic differences in the losses of organ-weight in chronic inanition as compared with acute inanition in adults (Jackson '15 a, '15 c).

Finally, it should be remembered that the results of the present paper, as well as those concerning adult chronic inanition in a previous paper (Jackson '15 c), are based upon the use of a diet wholesome and balanced, but insufficient in quantity. It is probable that more or less different results would follow from other forms of inanition, such as 'partial inanition' from a chemically defective or highly unbalanced diet. For example, Hatai ('15) finds a pronounced atrophy of the testis and other characteristic changes in albino rats whose growth had been retarded by a 'lipoid-free' ration. Bowin ('80), however, in dogs and rabbits fed dry food only (no water) found, with a loss of about 50 per cent in body-weight, the losses in organ-weight similar to those following total hunger.

SUMMARY

The principal results of the present paper may be summarized briefly as follows:

Young albino rats may be held at constant body-weight for considerable periods by underfeeding. The amount of food required for this purpose decreases as the experiment proceeds.

As to the body-proportions, the relative weights of the head, trunk and extremities remain practically unchanged during the experiment. There is apparently a slight increase in the head, counterbalanced by a corresponding decrease in the trunk and extremities, but the change is so slight as to seem of doubtful significance.

Of the systems—integument, skeleton, musculature, viscera and 'remainder'—there is but little change in the weights of the musculature, visceral group (as a whole) and 'remainder.' There is, however, a marked decrease in the weight of the integument, counterbalanced by a marked increase in the skeleton. Thus on the low plane of nutrition in the young body maintained at constant weight, the growth capacity appears weakest in the skin and strongest in the skeletal system. This is in striking contrast with the normal growth process of corresponding ages, during which the musculature increases with relatively great rapidity and the skeleton lags behind relatively.

The increase in the skeleton during constant body-weight appears to involve the ligaments as well as the cartilages and bones. The skeletal growth tends to proceed along the lines of normal development, as indicated by decrease in the water-content, and by formation and union of various epiphyses. Another evidence of the tendency to normal development of the skeleton is seen in the increased relative length of the tail as compared with the body-length. The teeth also continue to develop normally (formation and eruption of the third molars).

The individual viscera may be classified in three groups:

(1) There is during the maintenance of constant body-weight in young rats a well-marked increase in the weights of the eye-

balls, spinal cord, alimentary canal (both empty and including contents), testes, hypophysis and suprarenal glands. The suprarenals undergo sexual differentiation in weight (as occurs normally), but the hypophysis apparently does not.

(2) There is no marked change in the weights of the brain, heart, kidneys and epididymi. The liver is variable, showing a definite increase in the earlier periods, but a decrease later. The lungs show a slight decrease in the early periods, but not in later.

(3) There is a well-marked decrease in the weights of the thymus ('hunger involution'), spleen, thyroid gland and ovaries.

When the organs are similarly grouped according to degree of loss during chronic inanition in the adult (slight loss, loss proportional to body, and loss greater than body), many differences are found on comparison with the corresponding groups in the young during constant body-weight. This is explained as due to to the presence of both the growth tendency and the (more or less different) maintenance tendency in the young animals, whereas in the adult there is only the tendency to maintenance. Both the growth tendency and the maintenance tendency, however, show characteristic differences in the various organs according to nutritional conditions (normal nutrition, acute or chronic inanition).

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[*Reprinted from* SCIENCE, *N. S., Vol. XLII., No. 1093, Pages 819–828, December 10, 1915*]

OBSTACLES TO RESEARCH[1]

THE duty of the university to investigate the unknown as well as to teach the known is clearly evident. In the performance of this duty, the importance of research work is emphasized in many ways. Promise of productive scholarship is a leading qualification demanded in selecting members of the faculty. Encouragement and facilities for original work are freely provided. And yet we must confess that the outcome, broadly speaking, is somewhat disappointing, both here and in other universities. It is true that the results in some departments and in many individual cases are satisfactory. On the whole, nevertheless, considering our great opportunities, we seem to add relatively little to the sum total of human knowledge. Why? A recognition of the obstacles to research might enable us in some measure to overcome them. At any rate, the problem is worthy of our earnest and careful consideration.

Let us consider the problem from the biological point of view. The accomplishment of every human being (as of all living things) is the resultant of two factors: heredity and environment. In research work, as in all other lines of activity, the *limits* of possible achievement for each individual depend upon his innate talent, established through heredity. Within these limits, however, the *realization* of possibilities is conditioned by the environment. We must therefore distinguish clearly between (possible) capability or capacity and (actual) accomplishment in the field of original investigation.

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[1] An address delivered before the Minnesota Chapter of the Sigma Xi Society, October 21, 1915.

The first and most important obstacle in research work is accordingly the limitation of capacity, which is determined by heredity. Since it is now too late to quarrel with our ancestors concerning the matter, as individuals we may as well recognize this as an insurmountable obstacle. From the broad university point of view, this fundamental obstacle may be partially removed by great care in the selection of faculty members. Geniuses are scarce, however, and competition for them very strong; so it is inevitable that even in the strongest universities the faculties must be made up of men with varying degrees of innate talent.

But while our heredity is beyond our control, our environment is not. At least we can modify the environment to a considerable extent. And this is a fact of tremendous practical importance. After all, environment does play an important part in determining both the quantity and the quality of our performance in all lines, including research work. If the environment is sufficiently unfavorable, even the highest genius is sterile. Of two men with equal native ability, one with better opportunity may be far more richly productive than the other. It is a case of seed and soil. The result is determined by heredity plus environment; or perhaps better, heredity *times* environment.

Geniuses are sometimes able to accomplish a great deal, even in a relatively unfavorable environment; but fortunately research work is not a province reserved exclusively for genius. It is encouraging to most of us to remember that the army of investigation requires private soldiers, as well as officers of various grades. Even moderate capacity does not preclude research work of real value. As expressed by John Hunter:

> A man with a sufficient fund of knowledge, and a close application to one art or science, will make

great improvements in it though his talents may not be the best; or, in other words, though he be not a great genius.

Conquering the unknown in the field of knowledge is somewhat like civilization invading a new territory. A few bold and talented explorers may lead the way and blaze out new paths in the wilderness; but their excursions would be fruitless unless followed up by pioneer settlers, who by arduous labor develop the country and render its resources available for mankind. Moreover, even the explorer is in many ways largely dependent upon the knowledge and equipment furnished by others, his predecessors and his supporters.

Likewise, in the exploration of the field of knowledge, there is work for all. The history of science abundantly proves that brilliant discoveries and important generalizations usually rest upon a long series of accurate observations, requiring care and patience, but not great genius. A classical example is that of Kepler's laws of planetary motion, founded upon the extensive astronomical observations by Tycho Brahe. In biology, to substantiate and support the cell-doctrine of Schleiden and Schwann, and the doctrine of organic evolution of Darwin, has required an immense amount of patient labor by a multitude of observers during the past century. Other examples could easily be cited in various fields. The plodders as well as the geniuses should receive their due share of credit for the progress of science.

The production of research work of merit is thus within the capabilities of every one worthy of membership in a university faculty. Doubtless some who are talented predominantly as teachers should devote themselves chiefly to this field, and others are especially fitted for administrative work; but it is desirable that every one should participate to at least a slight extent in research work. As a matter of fact, we may go still further in urging that the *spirit* of scientific research should pervade *all* education, from the kindergarten to the university. Mankind in general is still far from appreciating the fact that the method of science is not a mysterious gift of genius, but a practical tool in the discovery of facts and in their application to the problems of everyday life. As Professor Remsen so aptly expressed it in his address at the dedication of the chemistry building of the University of Minnesota last year, the scientific method is essentially this: "First study the facts; then draw your conclusions from them."

From this point of view, all our problems thus become research problems; and education is able to teach us how to solve them efficiently in proportion to the extent to which training is provided in the methods of original investigation. Thus all education should provide training in scientific research, differing in degree rather than in principle as we pass from elementary to higher education. President Hill (in a recent commencement address at the University of Minnesota) has well said that "The teacher should arouse the spirit of discovery as the first step in the process of learning." A more general recognition of the significance of scientific research for education, a correction of the prevalent error that research is a matter concerning only a chosen few, would remove an obstacle which prevents a more generous support of higher scientific investigation.

While all instruction should be permeated with the research spirit, a conscious effort should be made, especially in the university, to single out as early as possible those students showing unusual talent for original work, and to give them particular aid and encouragement. We must constantly emphasize the necessity for recognition of unusual talent, since otherwise our entire time and energy will tend to be exhausted in caring for the larger number representing mediocrity. This subject is well discussed in a recent report of the subcommittee on the selection and training of students for research (Committee of One Hundred of the American Association for the Advancement of Science), published in SCIENCE, September 17, 1915.

It is, however, not my present purpose to consider the message of science for education in general, but rather to discuss the specific obstacles met by university workers in the field of original investigation. Since we can not change our heredity, possibilities for improvement must be found in the environment. What factors in our environment affect our scientific productivity?

We might classify the environmental factors affecting our research work in two groups: mental and physical. In the first rank, I would place the factors determining our mental attitude toward research. I suspect that investigation lags more frequently from lack of sustained interest than from any other cause. It is doubtless true that one is usually most interested in what one can do especially well. And research ability, as we have already noted, is largely a hereditary matter. Nevertheless, our mental attitude is unquestionably influenced in large measure by the opinion of our colleagues. Appreciation by one's fellows is a most powerful stimulus. Thus a general recognition of good research work will greatly encourage the worker to persist in spite of all obstacles. If Sigma Xi can succeed in establishing a more enthusiastic *esprit de corps* among investigators, it will greatly help the cause of scientific research.

The physical factors affecting research work are also of importance. The obstacles under this group include lack of material facilities, lack of time and lack of organization. Each of these may be briefly considered in turn.

The material facilities necessary for research include laboratories and equipment of various kinds, supplies, instruments, technical assistants, books, etc. It is customary to cite lack of adequate facilities of this kind to explain shortcomings in scientific productivity. And there is no doubt that more generous provision for these things would greatly facilitate many lines of research work. But, generally speaking, I believe that this factor is somewhat overrated. The man who does nothing because facilities are inadequate would usually accomplish but little even with unlimited resources. On the other hand, the man whose heart is in his research work will rarely fail to secure adequate support, if he perseveres and demonstrates his interest and capability.

Inadequate support of research work is sometimes ascribed to lack of appreciation on the part of university administrative officers, who control the purse-strings. This is usually an unjust accusation. University officers as a rule are keenly anxious to encourage and support research work, but they in turn are always more or less hampered by financial limitations. With the present evidently increasing popular interest in and appreciation of scientific work, however, we may confidently expect in the future more generous provision of funds available for this purpose. Even the "man in the street" can see how Germany has increased her efficiency by systematic encouragement of scientific research. America is likewise beginning to realize that this is not a luxury but a necessity, for which generous support must be provided.

Even more than lack of facilities, lack of time is an obstacle very frequently encountered by university research workers. Many university men are carrying a burden of routine teaching which, if well done, must greatly encroach upon the time absolutely essential for serious research work. In many cases, a considerable amount of routine administrative duties, committee work, etc., is added. Under these conditions, which shall be neglected—teaching, administrative work, or research? Or should one risk the danger of overwork by trying to keep up with all? Surely this is a question hard to answer. The proper solution is of course to provide a sufficient staff to handle the routine teaching and administration, and at the same time leave adequate time free for research. In a rapidly growing university, however, it is difficult to make this provision. But conditions are improving in this respect, and comparatively few men are so overburdened with routine work as to preclude a reasonable amount of time for research.

Lack of time for research work is often due not so much to the actual amount of other work as to waste of time. By carefully planning our university work, much time could be saved. There is too much "scatterment." All too frequently we allow minor routine duties to break in at all times. These minor details should be concentrated so far as possible at certain designated periods, so as to leave uninterrupted consecutive time free for research. A set of office-hours established and rigidly kept will gain a surprisingly large amount of time otherwise frittered away. Thus one serious obstacle to research may be readily removed.

Finally, I believe that another obstacle of importance in many cases is the lack of a proper organization of the research work itself. For the best results, careful, systematic planning is necessary. Too often investigation is taken up in a haphazard sort of way, which is likely to result in failure. While no rule can be made which will apply to all cases, it is certainly true that the topic to be investigated should be carefully considered before the work is undertaken. The literature should be scanned sufficiently to make sure that the contemplated problem has not already been solved, and to render available the experience of others in similar fields. Work should not be undertaken until the necessary facilities are assured to carry it through. In general, a broad fundamental problem of which successive phases may be worked out through a series of years will prove more profitable than a number of shorter, unrelated subjects of investigation. Wherever possible, cooperation with one's students or colleagues in research will usually yield better results, from the standpoint of economy in time and cost, than will individual efforts. Such matters may seem self-evident to some and trivial to others; but I feel sure that in many cases more attention to them would be well worth while. In short, system is as necessary for efficiency in research as in any other kind of work.

In conclusion, the main points may be emphasized as follows: Obstacles to achievement in research are due partly to inherent or hereditary limits of capacity, and partly to environmental factors. The latter, which are to some extent within our control, include factors determining the mental attitude, which is of primary importance. The remaining factors include the material facilities, increased support for which depends chiefly upon better appreciation by the public of the value of scientific work. Lack of time is often another important obstacle, which in part may be overcome by a more economic arrangement of routine duties. Finally an obstacle in many cases is the lack in the research work itself of systematic planning and organization, which is necessary for the highest efficiency.

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Reprinted from THE ANATOMICAL RECORD, Vol. 8, No. 4
April, 1914

THE NERVUS TERMINALIS IN MAN AND MAMMALS[1]

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NINE FIGURES

It is over nineteen years since Pinkus ('94) first called attention to a 'new nerve' attached to the telencephalon of Protopterus, and thirty-five years since the first record of this nerve having been seen in a shark (Fritsch '78). The forms in which this nerve has now been recorded and its chief characters have been briefly summarized in the writer's previous communication ('13). In that paper the existence of a true nervus terminalis in human and certain mammalian embryos was clearly established. At the same time McCotter ('13) pointed out the existence of the nerve in the adult cat and dog. Huber and Guild ('13) have since studied the peripheral relations of the nerve in the rabbit in late foetal stages and during the first six days after birth.

The purpose of the present note is to call attention to the presence of the nervus terminalis in certain other adult mammals in the hope that a larger number of workers may undertake the study of its central and peripheral relations. At the present time it is clear that at least a part of the nerve is distributed to the mucosa of the nasal sac and in mammals accompanies the vomero-nasal nerve. A part of the nerve, however, in mammals clearly goes beyond the limits of the vomero-nasal organ. In the rabbit it spreads over a rather wide area of the nasal septum (Huber and Guild). The nerve is usually accompanied by ganglion cells, which Brookover ('10) believed to be sympathetic in character. Huber and Guild incline to the same conclusion. Although the central relations of the nerve have been studied by special methods by Herrick ('09), Sheldon ('09) and McKibben

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[1] Neurological Studies, University of Minnesota, no. 19, November 15, 1913.

('11) it is still not known whether its fibers are all afferent, or whether some or all of the fibers arise from cells within the brain. In the latter case they might be considered preganglionic fibers of the sympathetic system. The present state of our knowledge suggests the probable presence of both afferent and efferent components in the series of vertebrates. The wide-spread presence of the nerve in adult mammals, including man, should add interest to the study of its relations.

The pig. In my previous communication it was stated that the nerve had not been seen in 73 and 90 mm. pigs. Since then it has been found by dissection in numerous pig foetuses ranging from 50 mm. to full term. The brain of the adult pig has not been examined.

The horse. Figure 1 shows the proximal portion of the right nervus terminalis in the brain of an adult. The figure shows a small portion of the basal surface of the brain between the olfactory trigon and the median fissure. A part of the anterior cerebral artery is seen in the right hand part of the figure. The plexus of small vessels lies immediately upon the brain substance, the nervus terminalis lies outside of them and is in turn covered by the pia. The nerve has about fourteen rootlets which enter the brain along the rostral and medial border of the medial olfactory tract. The rootlets unite by twos and threes and eventually form a common nerve trunk. Upon the largest one of three main roots into which the rootlets unite, as seen in the figure, there is an obvious ganglion. The nerve trunk extends forward nearly parallel with the olfactory peduncle to a point opposite the rostral border of the olfactory bulb, where it is lodged in the pial septum between the hemispheres. Here the nerve had been cut off in removing the brain from the skull.

The nerve of this side was removed after drawing and cut into three pieces for staining. The distal piece was treated with vom Rath's picro-osmo-palatino-acetic mixture, cleared in cedar oil and mounted in damar. It contains three fairly well medullated fibers and seven or eight fibers which were lightly and irregularly blackened. The middle piece was stained in a mixture of nigrosin and acid fuchsin but a differential staining of nerve fibers and connective tissue was not obtained. The proximal piece included a part but not all of the ganglion seen in figure 1. The piece was stained in neutral red. A number of cells were found scattered along this piece and the portion of the ganglion consisted of about twenty closely packed cells varying in size. All the cells had large nuclei with prominent nucleoli. Although the Nissl bodies were not clearly stained, owing to unsatisfactory fixation, there is no doubt that the cells are nerve cells. Two nerve cells were seen also in the piece stained by nigrosin.

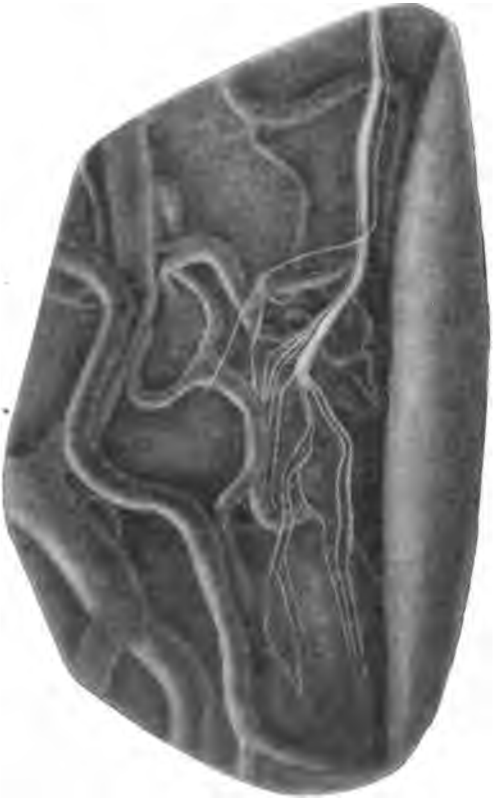
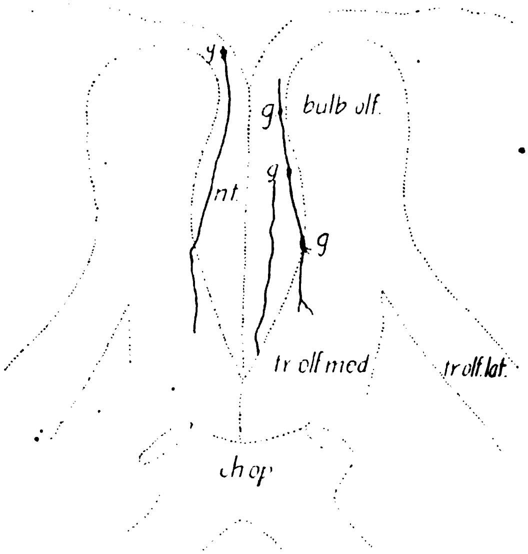


Fig. 1 Root of the nervus terminalis in the horse, right side. Description in text.

The nerve on the left side of this brain is similar to this although it differs in the number and arrangement of rootlets.

The sheep. Three brains of the adult sheep have been examined. The nerve was not found in the first but was present in the other two. In one of these brains (fig. 2) there was a single strand on the right side and two strands on the left. One of the latter strands presented three conspicuous ganglion-like enlargements. Upon staining and mounting these proved to be true ganglia. The nerve of the right side contained single ganglion cells scattered along its course, two collections of six or eight cells each and a ganglion at its distal end larger than any one of the three on the left side. A piece of the left nerve, treated in vom Rath's fluid, showed a single lightly medullated fiber.

The porpoise. I am indebted to Mr. W. F. Allen of this laboratory for the brain of a porpoise (Phocaena) preserved in Bouin's fluid. The brain of the porpoise has a very broad, rounded frontal lobe (fig. 3), the optic tracts diverge very widely and the anterior perforated space is greatly elongated from side to side. In the absence of the olfactory bulb and peduncle the topographical relations in this part of the brain must be based chiefly on the extent of the anterior perforated space. Upon the basal aspect of the frontal lobe there are seen beneath the pia seven slender strands which converge forward to a point corresponding as nearly as may be judged to the point at which the nervus terminalis was cut off in the horse's brain. Here likewise the nerve had been cut in removing the brain. Proximally the strands enter the brain over a wide area. The most lateral one enters the lateral part of the anterior perforated space. The most medial strand runs along the median fissure and bends up on the medial surface to penetrate the brain in the fissura prima on this medial surface (fig. 4). Two strands follow the anterior cerebral artery in the median fissure and bend laterad with it almost in contact with the rostral surface of the optic chiasma and enter the brain in the depth of the fissura prima on the basal aspect. The nerves of the left side have been described and drawn; those of the right side have a similar arrangement. The nerves are relatively larger than in the horse and are more closely applied to the brain surface throughout their course. They differ also in that the rootlets are flattened strands which run for a longer distance before uniting.



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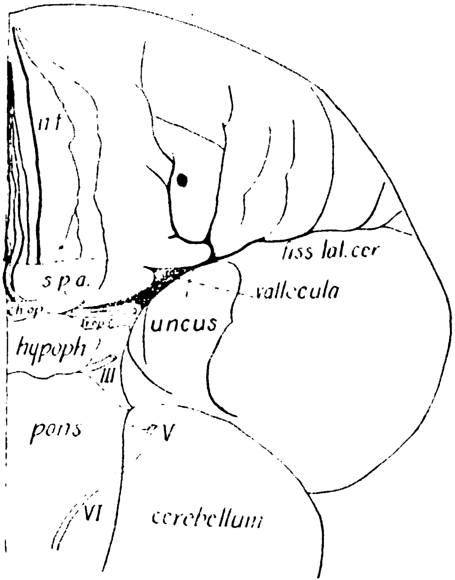

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Fig. 2 Nervus terminalis in the sheep; *g*, ganglia; *ch.op.*, optic chiasma.

Fig. 3 Basal aspect of the brain of the porpoise; *s.p.a.*, substantia perforata anterior. The broken line bounding this rostrally marks a small sulcus occupied by a blood vessel. Three of the rootlets penetrate the brain beneath this vessel. *X* indicates point at which a root was broken in dissection.

The seven-months foetus had been in formalin for at least six years and had not been fresh enough for histological study when preserved. The brain was rather soft and the tissues tough, so that the attempt to trace the nerve into the nose had to be given up. The brain was carefully removed and upon examination under the Greenough binocular, two transparent nerve strands were seen (fig. 6) upon the orbital surface of the gyrus rectus which were cut off opposite the anterior end of the olfactory bulb as in the forms above described. The apparent change in the position of the nerve since the five month stage is due to the rapid development of the frontal lobe, which has expanded mesad producing a gyrus rectus medial to the olfactory peduncle. The pre-chiasmatic space is filled with a gossamer-like fibrous tissue which had to be removed patiently in order to follow the strands to their point of entering the brain. On the left side one of the strands pierced the rostral border of the medial olfactory tract near the trigon. The other strand divided into two rootlets which ran deeper into the fissura prima (fig. 6). On the right side the nerves were not fully dissected, the anterior cerebral artery being left in position to show the relations.

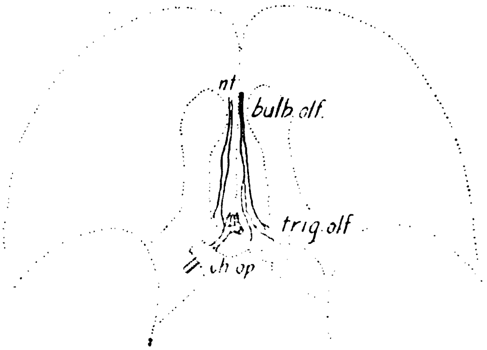


Fig. 6 Seven months human foetus, basal surface of the frontal lobes showing the nervus terminalis.

In the full-term foetus the attempt was made to trace the peripheral course of the nerve. The dissection was made from the face in order to expose the orbital surface of the frontal lobe. The nerve of the left side was found on the gyrus rectus as in other cases. It was a single thick strand readily visible to the naked eye. Traced forward it entered the median fissure opposite the anterior end of the olfactory bulb. Curving somewhat dorsad in the fissure the nerve makes a gentle curve ventrad again and at the same time leaves the surface of the brain, with which it is in contact, and enters the pia mater. At the same time the nerve divides into several strands which flatten out like a fan. These thin flat strands pierce the pia and enter the tissue of the cribriform plate close to the median plane among the most anterior strands of the olfactory nerve. Here the connective tissue was so tough, owing to the formalin preservation, that the thin strands could not be followed far. Some of them were followed without doubt into the septum, and some appeared to go toward the lateral wall of the nasal chamber, but this was uncertain. The point at which these strands pierce the pia mater is the place where the nerve is cut off when a brain is removed from the skull. The fact that at this point the nerves in the adult brains dissected were either within or very near to the median fissure and imbedded in the pia, suggested the possibility that the nerves might be distributed to the meninges, but the dissection of this specimen was carried far enough to show conclusively that they go down into the septum, and to explain their position in the adult brain.

The brain of a baby of four months showed two strands on the left and three on the right. On both sides the roots entered the brain beneath the medial and rostral border of the medial olfactory tract. The nerve of the left side was stained and mounted, but no ganglion cells were found.

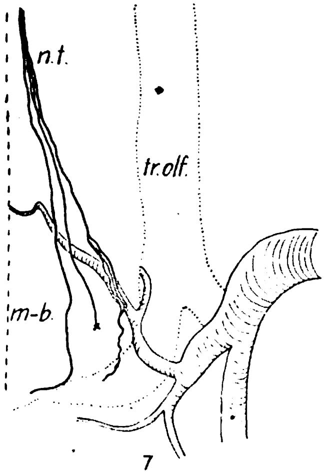
Fourteen adult human brains have been examined and the nervus terminalis found in all. In most cases the nerve is visible under the lowest power of the Greenough binocular without any dissection. It is only necessary that the pia mater shall be intact in the region between the olfactory peduncles and rostral to the optic chiasma. In only one of the fourteen brains was the nerve so small as to be at all difficult to follow. The nerve strands lie just beneath the pia and are visible through it because slightly more whitish opaque than the pia. They are distinguished from small blood vessels because the vessels, even when apparently empty, have a slightly yellowish color. Moreover, small blood vessels are readily traced to the larger ones from which they arise. It is more difficult to distinguish the nervus terminalis from thin bands of connective tissue which lie in or beneath the pia. Most of the connective tissue strands in this region are inserted in the thick tissue surrounding the anterior cerebral artery or some of its branches in the median fissure, from which they stretch obliquely forward and laterad. The connective tissue strands can be detected by pulling this way and that upon the pia with fine forceps. The strands of connective tissue will be distorted and the appearance of strands will be produced parallel with the direction of tension, while the nerve strands, if present in the area pulled upon, will not be distorted, nor obscured. When the pia mater is pricked through, lifted up with forceps and dissected away it is found that the nerve strands lie beneath the pia but are attached to it more or less closely. The attachment is by means of slender connective tissue strands and in some instances by small bands which pass beneath the nerve in the form of loops or straps. In some cases the length of the nerve was drawn through one of these loops in order to free it from the pia. With a little care the nerve is separated from the pia and a few minutes suffice to expose the nerve through the greater part of its intracranial course. The dissection of the roots where they bend around the heel-like convexity of the gyrus rectus just in front of the optic chiasma requires more care, especially when the nerve divides into several slender rootlets.

Figures 7, 8 and 9 show the course of the nerve in three adult brains. It runs over the orbital surface of the gyrus rectus and

Fig. 7 Part of the basal aspect of the left frontal lobe in the adult human brain. The blood vessel is a small vein greatly distended with blood. *X* marks a root broken in dissecting.

Fig. 8 Basal aspect of the right frontal lobe in the adult human brain. Anastomosis of rootlets.

Fig. 9 Similar to figure 8. Nervus terminalis, a single strand with five rootlets on the surface of the medial olfactory tract.



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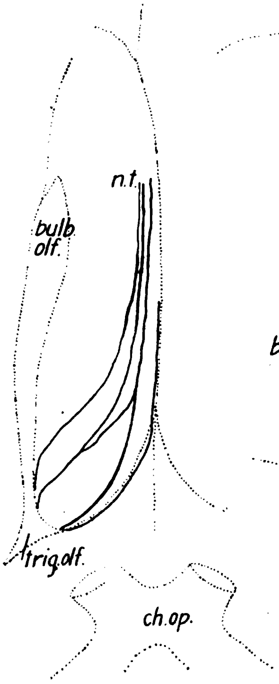

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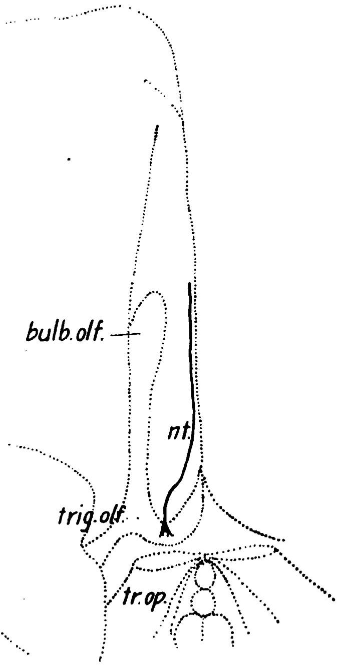

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enters the median fissure or lies near it at the level of the anterior end of the olfactory bulb, where the nerve is cut off in removing the brain. As seen in the figures, the nerve in these cases consisted of one, two, three or four strands. In one case (fig. 7) one of the strands divided into three strands which again united into one. In another case (fig. 8) a slender strand crossed obliquely from one of the main strands to another. It is entirely possible that slender strands would be overlooked or destroyed in dissecting and two or three minute strands were seen which are not included in the drawings. In the fourteen brains the type of nerve most frequently met with is that shown in figure 8. In one case the subdivision and reuniting of strands seen in figure 7 was present, together with a large number of very slender strands which ran along the medial border of the olfactory peduncle and bulb. When the nerve consists of a single strand, as in figure 9, it is large enough to be seen and dissected without the use of a lens. In one case it appeared larger than some of the rootlets of the IX and X nerves; but, being a broad thin band, it probably contains fewer fibers than those rootlets.

The point of attachment of the roots to the brain varies considerably. In all cases thus far examined it is in the region of the basal end of the fissura prima, either upon or in front of or behind the medial olfactory tract. In figure 8 the roots are seen converging toward the olfactory trigon, where they seemed to dip beneath the anterior and medial border of the olfactory tract. In figure 9 the single root divides into five rootlets which pierce the medial olfactory tract. The two roots of the other side of this brain had the same position. Two of the three roots in figure 7 have nearly the same position but one of them passes farther toward the median plane.

In position these strands correspond very closely to the nervus terminalis of lower animals and of human embryos previously described. Proof of the nervous character of the strands was sought, however, by removing and staining some of them. In one brain the nerve was found on only the right side. Its two rootlets entered the brain just at the rostral border of the medial olfactory tract where that bends from the orbital to the medial surface. The roots were pulled out and the entire nerve was stained in neutral red and mounted in damar. It consists of some fifteen or sixteen small bundles of non-medullated fibers and has imbedded in its course about twelve ganglion cells. These cells occur singly or in twos. There is no larger collection. Each cell has a large nucleus and prominent nucleolus and stains deeply with neutral red. Unfortunately the material was not fresh enough to give a good stain of the Nissl substance.

The nerve on the right side of the brain from which figure 9 was taken was removed and stained as was also the left nerve of the four months baby brain, but no nerve cells were found in either. From the brain shown in figure 8 the nerve of the left side was stained in neutral red, while that of the right side was treated with vom Rath's fluid and afterward stained with carmalum. Neither medullated fibers nor nerve cells were found in this case. The largest nerve found was treated in the same way and teased out carefully. After clearing and mounting in damar there were found four ganglion cells surrounded by numerous sheath-cell nuclei. Two cells were in the middle part of the nerve, two at the distal extremity. No medullated fibers were found. Failure to find ganglion cells in some of the other cases may have been due to the fact that the nerves were not as well teased out.

From the above facts it appears that a nerve corresponding to the nervus terminalis of lower vertebrates exists in adult man and several other mammals. This nerve contains some medullated fibers at least in the sheep, horse and monkey, and in the monkey these fibers increase in number distally. In the sheep, horse, monkey and man the nerve contains ganglion cells in at least some individuals. In the sheep and horse there are distinct ganglionic enlargements of the nerve. The failure to find ganglion cells in the other cases here reported has little significance, since only the intra-pial course of the nerve could be examined. The probability that ganglion cells would be situated farther distally is suggested by the condition in the rabbit (Huber and Guild) and by the presence of a large ganglion in the sheep (fig. 2) just at the point where the nerve pierces the pia, rostral to the olfactory bulb. The presence and large size of the nervus terminalis in the porpoise serves to emphasize its independence of the olfactory nerve, which has been abundantly established by previous work.

The nerve has usually been regarded as a vestigeal structure, but it is an interesting fact that it is larger in man than in many fishes and amphibians. The question suggests itself, for how many million years has the nerve persisted in a vestigeal condition? Further studies are desirable upon the central relations of the nervus terminalis and upon its structure and distribution in the nasal septum.

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Reprinted from THE JOURNAL OF COMPARATIVE NEUROLOGY, VOL. 25, No. 3
June, 1915

A TRACTUS OLFACTO-TEGMENTALIS IN THE HUMAN FETAL BRAIN[1]

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NINE FIGURES

It is commonly taught that the most direct pathways over which impulses aroused in the olfactory centers may reach the motor centers are the two following:

a. From the olfactory centers in the forebrain to the tuber cinereum or the corpus mammillare, and from these to the tegmentum or possibly to motor centers directly;

b. From the forebrain centers to the nucleus habenulae, thence over the bundle of Meynert to the interpeduncular nucleus and thence to motor centers over paths not well known.

The following description adds to the evidence that a connection exists between the olfactory and lower centers without interruption in the diencephalon.

The brain studied is that of a fetus 145 mm. in length, crown-rump measurement. It was received fresh and fixed in 15 per cent formalin. Sections were cut transverse to the long axis of the hemispheres and lengthwise of the brain stem, 50 microns in thickness, and stained with Delafield's hematoxylin. The preservation proved to be excellent.

The brain at this age is largely composed of cell-masses which are deeply stained. The fibers take little stain and where definite bundles are present, these stand out sharply among the cell-masses and neuropile. Certain well known tracts, such as the bundles entering into the anterior commissure and the fornix, are very clearly defined in this brain.

In a section passing just rostral to the anterior commissure (fig. 4) we see on the right side the precommissural fibers of the

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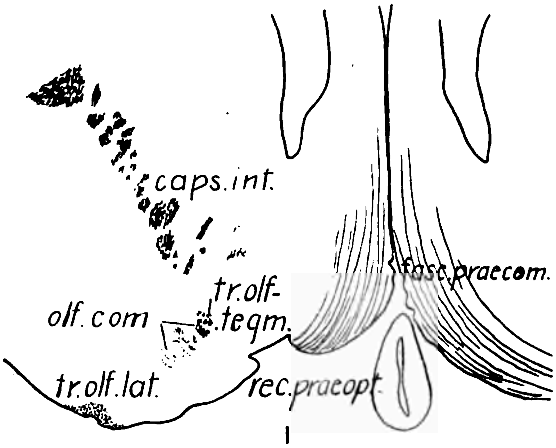
[1] Neurological Studies, University of Minnesota, No. 20, April 1, 1915.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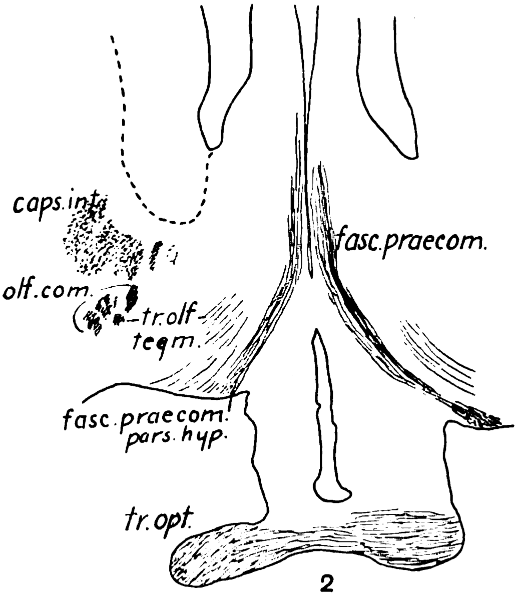
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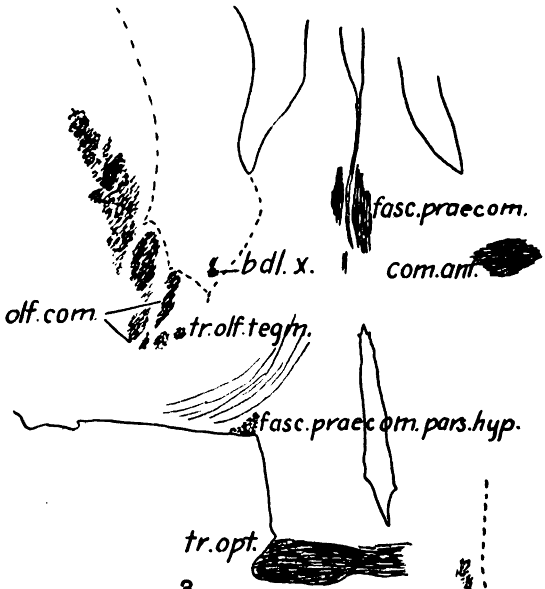
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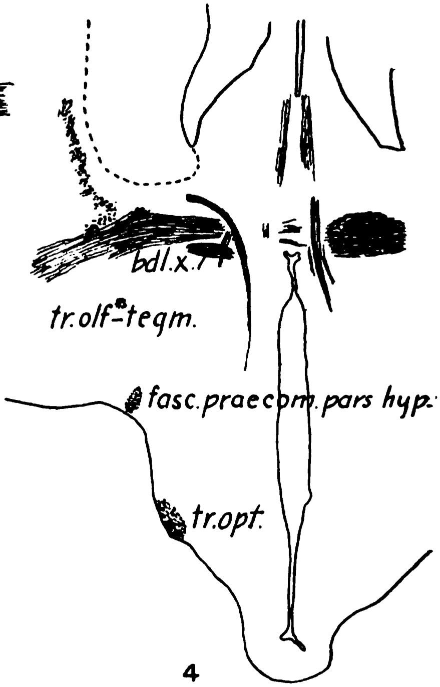
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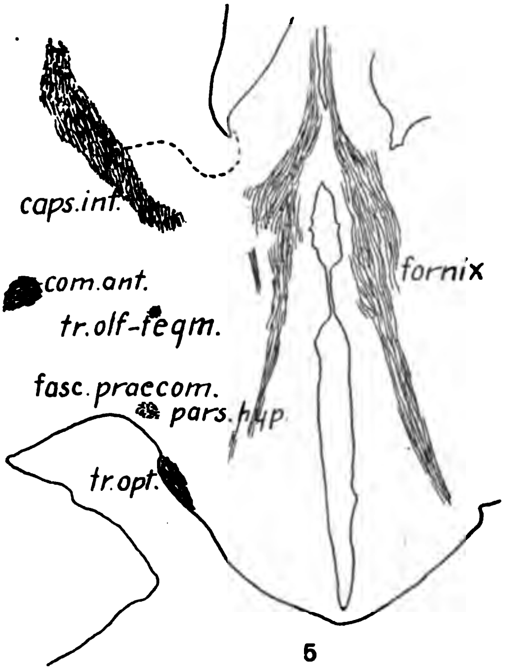
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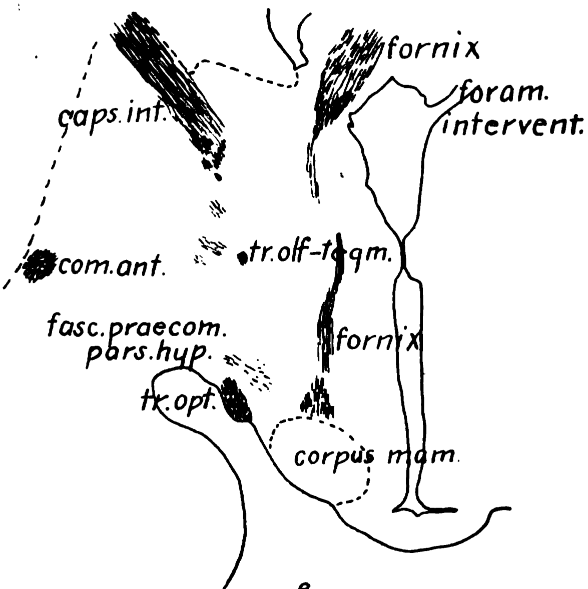
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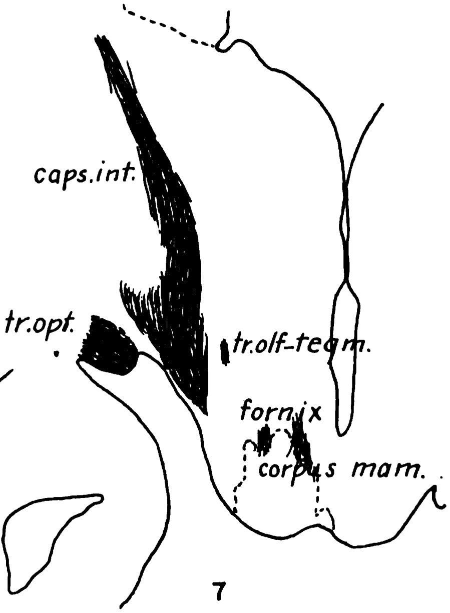
fornix crossing the anterior commissure vertically. On the left side appear a larger and a smaller commissural bundle. The smaller (*bdl. x*) is one of the olfactory bundles of the anterior commissure which crosses the mid-line independently of the main body of the commissure. Crossing these two bundles in a sweeping curve is a small bundle which runs down into the tuber cinereum. When this small bundle is traced rostrad it joins the smaller commissural bundle (fig. 3). Hence, a small bundle separates from the olfactory commissure, passes over the anterior commissure and enters the tuber cinereum. This condition has not before been described so far as the writer is aware.

In the section described there appears beneath the limb of the anterior commissure a fair sized bundle almost round in transverse section (*tr.olf-tegm.*). When this bundle is traced rostrad it is found that it intermingles with the olfactory bundles of the anterior commissure and has a commmon origin with these. This is illustrated in figures 1, 2, and 3. While some fibers may enter these bundles from the olfactory tract, there can be no doubt that most of the fibers are directly related to the olfactory tubercle and adjacent secondary olfactory centers.

The course of this bundle caudal to the level of the anterior commissure is shown in figures 5, 6, 7, 8, 9. The bundle is symmetrical on the two sides, but only the left side is drawn. It runs along the inner face of the internal capsule and descends somewhat into the hypothalamus until it comes to lie between the terminal portion of the fornix and the internal capsule (fig. 7). Beyond the mammillary body it rises again and gains a position dorsal to the medial border of the crus (fig. 8). The bundle then continues between the substantia nigra and the nucleus ruber and divides into several fascicles. These fascicles while continuing spinalward turn rather rapidly dorsad and somewhat laterad. They become imbedded in small masses of cells and are lost just at the upper or anterior border of the pons (fig. 9). One fascicle seems to enter the dorsal part of the pontile gray (fig. 9). One or two fascicles run much farther dorsad than the others, but do not approach so near the brachium pontis.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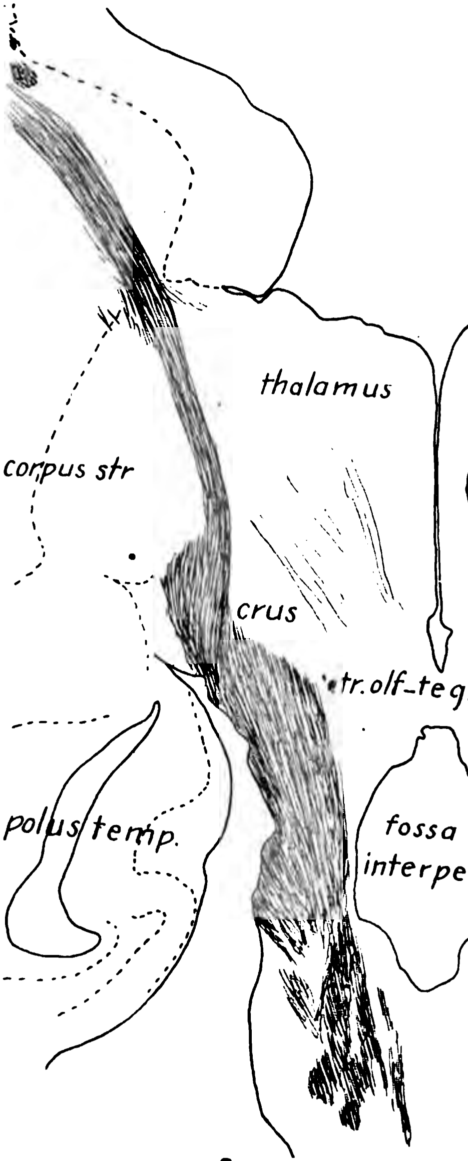
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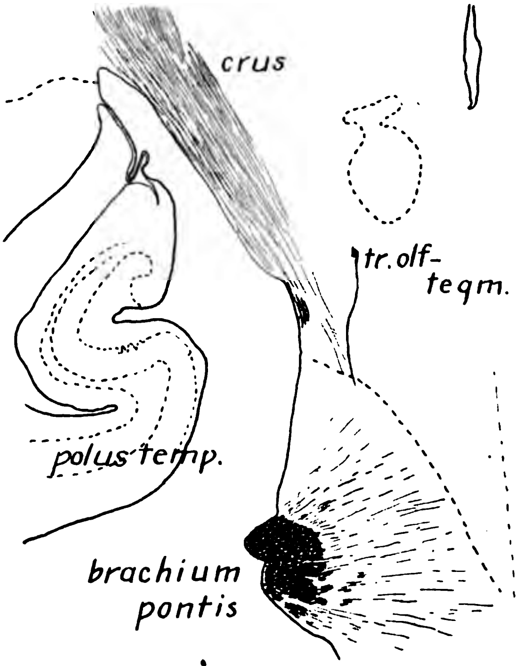

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The bundle described is interpreted as a descending tract from the olfactory centers to the motor-correlation centers in the tegmentum at the level of the isthmus. Such preparations give no direct evidence, of course, as to the actual origin and endings of the fibers. At their rostral end the fibers of this bundle are indistinguishably intermingled with the fibers of the olfactory commissure which we know arise from the olfactory centers. At its caudal end the fibers spread widely in the tegmentum. This renders it very improbable that the fibers arise in any of the sensory nuclei, such as the nucleus of the trigeminus. Its behavior rather suggests the breaking up of a bundle at its place of ending.

From the facts just stated the writer can see no reason for comparing this with the quinto-frontal bundle described by Wallenberg. That bundle is supposed by Edinger (Vorlesungen, 1908) to carry impulses from the sensory nucleus of the trigeminus to the tuberculum olfactorium, which serves as a correlation center for oral and nasal sensory impulses (muzzle-sense). If this were the function of the tuberculum olfactorium, one would expect that in forms like man, in which the olfactory apparatus is reduced and the oral sense is very important, the tubercle would be large relative to the other olfactory centers. Such is not the case. On the other hand the forms in which the tuberculum is large are those in which the olfactory apparatus is very large (Ornithorhynchus, Didelphys, dog).

There is more reason for comparing this with the descending bundle mentioned by Edinger (1908, pp. 123, 286) as arising in the lobus parolfactorius and running through the dorso-lateral part of the oblongata, to the cervical cord, tractus fronto-bulbaris. Edinger's lobus parolfactorius is a large, illy defined region "which includes the whole base of the brain" (p. 252), and there is nothing to indicate from what part of this area the tractus fronto-bulbaris arises. Also, it seems fairly certain that the bundle above described ends largely or wholly at the level of the isthmus. It is quite possible that some of its fibers pass on to the cervical cord and that these constitute the tractus fronto-bulbaris of Edinger.

Figures 2 to 6 show another bundle of considerable interest which is clearly defined in this series. This is the bundle of precommissural fornix fibers which enter the hypothalamus (*fasc. praecom. pars hyp.*). These constitute the most compact bundle in the system of vertical fibers in the "septum" rostral to the anterior commissure (fig. 2). The bundle is conspicuous in the sections back to the level of the optic tract, which it crosses to enter the lateral wall of the tuber cinereum. Whether the fibers reach the mammillary body can not be determined in this specimen.

Reprinted from THE JOURNAL OF COMPARATIVE NEUROLOGY, Vol. 25, No. 5
October, 1915

THE CELL MASSES IN THE FOREBRAIN OF THE TURTLE, CISTUDO CAROLINA[1]

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SIXTY FIGURES

In continuation of work upon the forebrain, it is the writer's intention to study the origin, growth and differentiation of the general pallium in the series of vertebrates. In doing this it will be necessary to keep constantly in mind the relations of the general pallium to the hippocampal formation, which has been described in earlier papers. The history of the amygdaloid complex and of the pyriform lobe is also intimately concerned. Finally, the position and arrangement of the fiber tracts connecting the hemispheres with the brain stem bear an important relation to the morphology of the cell masses in the forebrain.

In order to be able to compare given brain structures in lower and higher vertebrates it is necessary to have a description of the brain of some intermediate form in which the more common ichthyopsid characters can be recognized and in which at the same time the hippocampus and general pallium are present. For this purpose the brain of the turtle has been chosen and it serves admirably because of the relationship of the chelonia to the ancestors of mammals. The present paper will be confined almost wholly to description, the review of literature and the discussion of hypotheses being deferred to later papers.

METHODS

The results here presented have been obtained chiefly from the study of cell preparations. The results from the study of fiber tracts, although used in connection with this work, will be reported only briefly. The object has been to define the aggregations of neurones which there is reason to think are concerned with specific functions and to determine the position, extent and limits of these cell masses. Criteria depended upon are the position, size, form and grouping of the cells, their relation to fiber tracts and their relation to neighboring cell-masses. The more important aggregations of neurones are either separated from neighboring masses by cell-free zones, or they present a sudden transition to other masses whose cells differ markedly in size, position, staining qualities, etc. Sometimes sulci mark the lines of demarcation. Typical sections have been drawn to show the arrangement of cells in various regions and the lines of demarcation between the aggregations of neurones. To gain an understanding of the morphological relations, dissection and modelling have been practiced. After the various cell masses had been studied and their limits determined, a model was made of the right hemisphere together with a small adjacent part of the diencephalon. In making the plates for this model the limits of the several cell-masses were drawn under the projection microscope. The plates were then cut along these limiting lines but the parts not separated from one another. The model was then piled and fastened together as a whole and the several parts separated at last along the lines of the cuts. In this manner it was possible to pile the model accurately in the form of the control brain and since each part was strongly fastened together there was no distortion of the parts.

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[1] Neurological Studies, University of Minnesota, No. 21, July 20, 1915.

I am indebted to Dr. Wm. F. Allen for the beautiful photographs of this model.

DESCRIPTION

As is well known, the turtle has fairly large hemispheres whose caudal poles lie at the sides of the mesencephalon. The disposition of the hippocampal formation and the olfactory tubercle in the medial wall have been described in a previous paper. Other features in this wall will be taken up in later sections. The lateral wall shows a broad depression where the hemisphere connects with the brain stem which at once suggests comparison with the insular region in the mammalian brain (fig. 1).

The dorsal border of this depression is formed by a low ridge which extends rostrally into the lateral wall of the olfactory bulb. This ridge is traversed by the lateral olfactory tract and is therefore to be compared with the pyriform lobe of the mammalian brain. Near its rostral end it presents an apparent thickening which causes a decided elevation of the surface (figs. 5, 26). The sulcus which bounds the pyriform lobe dorso-laterally varies in depth in different individuals, but is always well marked in its rostral portion. It is the fissura rhinalis.

The insula-like region is clearly seen in the entire brain to be trasversed by bundles of medullated fibers (fig. 1). Sections show these to be a continuation of the crus cerebri (figs. 32, 49), and therefore comparable to the capsula interna. The absence of a cortical layer covering the internal capsule shows that this does not correspond to the insula. It is the corpus striatum which retains a condition analogous to that seen in the fish brain and is not yet covered in by pallium.

Caudal to the striatum there is a well-developed occipito-temporal pole whose presence adds to the similitude of the turtle's brain to that of a mammal. The pyriform lobe appears to blend with the occipital lobe. Along its lower border is a groove which at the rostral end is the sulcus endorhinalis. Behind the striated area a deep groove continues for some distance toward the caudal pole. This groove will be called the fissura amygdaloidea. A part of the area appearing below it in lateral view is occupied by the amygdaloid complex (fig. 5). The reason for calling this the amygdaloid fissure, however, is that it represents the line of infolding of an important body which becomes in mammals a part of the amygdaloid complex.

Rostral to the striated area appears the smooth lateral surface of the tuberculum olfactorium and adjacent olfactory area, including the lateral portion of the homologue of the anterior perforate space. Between this region and the pyriform lobe rostral to the striatum is the sulcus endorhinalis (figs. 19 to 26).

The vault of the hemisphere is occupied, as we shall see, by the general pallium. It is very evident that if the expansion of the general pallium were to push the pyriform lobe ventro-laterally until it was driven down nearly upon the basal surface and until the migrated general pallium itself had covered over the striatum, there would be produced a fairly exact counterpart of the primitive mammalian brain. This is the condition of the brain of an opossum or rabbit, in both of which the lateral (Sylvian) fissure is little deeper than the analogous striatal depression in the turtle's brain. The further expansion of the frontal and caudal poles produces the opercula bounding the lateral fissure.

On the dorsal surface just behind the olfactory peduncle occurs a slight rounded elevation (figs. 3, 25) which forms a part of the pallial thickening to be described below.

On the medial surface of a brain from which the stem has been cut away and the choroid plexus removed (fig. 3) it is seen that a wide chorioid fissure extends caudad in a simple curve from the interventricular foramen into the temporal pole. On removing the medial wall of the hemisphere there are exposed certain ridges which constitute some of the most characteristic features of the reptilian brain (figs. 4, 10). Three main bodies are to be distinguished: a *dorsal ventricular ridge*, the largest and most prominent; beneath it and extending farther rostrad, the *striatum;* and rostrally in the dorsal wall a smaller *pallial thickening.*

Three longitudinal ventricular grooves are to be distinguished: dorsal, middle and ventral. The dorsal ventricular groove is a very deep groove between the dorsal ridge and the pallium (figs. 4, 11 to 22). The middle groove runs beneath the dorsal ridge from the amygdaloid region forward into the olfactory bulb. A bifurcation of the caudal end of this groove and its significance will be mentioned later. The ventral groove is medial to the striatum. It dips very deep into the olfactory tubercle but is shallow rostral and caudal to this level.

The term epistriatum was first used by Edinger to designate a body in the reptilian forebrain to which C. L. Herrick had traced a large part of the olfactory tract. In the 1904 edition of Edinger's textbook this body is labelled epistriatum in figures 117, Varanus; 122 and 123, Schemata; 125b, lizard. This usage is followed by de Lange in a recent paper on Varanus. In Edinger's figure 121 the term epistriatum is applied to an entirely different body in the turtle, the dorsal ventricular ridge of the present paper. In figure 125a, which also represents the turtle, this ridge is called mesostriatum and the term epistriatum is applied to the pallial thickening. Since the structure to which the term epistriatum was first applied does not appear as a special body or ridge in the turtle brain and since the author of the term uses it for at least three different bodies in the reptilian brain, the term will not be used in the present paper. The use of purely descriptive terms will help to avoid confusion.

In the dissected brain or in the model from which the hippocampal formation has been removed (figs. 4, 10) it is seen that the dorsal ventricular ridge bends down into the temporal pole. Here it enters into close relations with the amygdaloid complex which must be discussed later.

FORMATIO OLFACTORIA

The formatio olfactoria is that portion of the forebrain which receives fila olfactoria and contains mitral cells and glomeruli. It forms the rostral part of the olfactory bulb and extends farther caudally on the dorsal than on the ventral surface. Thus the peduncular constriction is placed obliquely, as is evident in figures 3 and 5. The bulb in its rostral part is nearly triangular in cross section (fig. 30). The olfactory nerve divides into distinct ventral and dorsal roots. The ventral spreads upon the dorsal surface of the bulb and enters the brain farther rostrally than does the dorsal root. The ventral root spreads over somewhat more than the lower half of the medial and lateral walls (fig. 30) and the rostral surface. The dorsal root continues over the bulb as a high ridge and spreads out in the dorsal wall in its caudal part. At its greatest extent, near the peduncle, this root extends half way down on the medial and lateral surfaces. The areas in which bundles of fila olfactoria are evident are shown in figures 28, 29, 30. It is certain, however, that many fila extend either in small bundles or singly some distance beyond these limits before ending in relation with neurones of the formatio olfactoria.

The limits of the formatio olfactoria can be determined more accurately by a study of the form, size and grouping of the cells. Throughout all the area where fila olfactoria are visible, the wall of the bulb consists of a thick layer of granule cells adjacent to the ventricle, and a fiber layer of varying thickness in the midst of which are scattered large and small mitral cells. In the areas not covered by bundles of fila olfactoria the mitral cells are less numerous and sometimes absent (figs. 28, 29, 30).

The deep granule cells are very numerous, closely crowded and sometimes arranged in irregular concentric layers. Golgi sections show that they possess two or more long slender dendrites radially placed and that the dendrites interlace with those of the mitral cells in the glomeruli. These cells are therefore true olfactory receptive cells, comparable to the deep cells of the olfactory formation of fishes (Johnston '01). The granule cells form a dense thick layer surrounding the ventricle throughout the whole extent of the bulb (figs. 28, 29, 30) and constitute the greater part of its volume. The groove or constriction which marks the olfactory peduncle follows accurately the caudal border of this granule cell layer. In the lateral wall the pyriform lobe and the olfactory tubercle push forward somewhat and the peduncular groove presents a V-shaped bend forward (fig. 5), but everywhere the groove faithfully indicates the boundary between primary or bulbar structure and the secondary centers or olfactory lobe.

The layer of mitral cells and of small brush cells is not as extensive as that of granule cells. In the rostral half of the bulb this layer is continuous around the periphery of the section, but the cells are less numerous in the lateral and medial angles where the fila olfactoria are not evident. In the caudal part of the bulb the corresponding areas become quite free from mitral cells for some distance (fig. 29, 31). The distribution of the mitral cells is determined by their relation to the fila olfactoria. Golgi preparations show that the dendrites of mitral cells and of the small brush cells penetrate the bundles of fila and ramify richly, thus transforming the bundles into elongated glomeruli or series of glomeruli. A brush often lies on the surface of one of these bundles and its dendrites wrap around and penetrate the bundle. The formation of glomeruli in the course of large bundles of fila is a condition similar to that in Petromyzon described by the writer (1902). In addition to these large glomeruli are numerous smaller ones formed by the dendrites of small brush cells and of granule cells.

The formatio olfactoria consists then of (1) an enormous number of granule cells whose bodies form the dense peri-ventricular layers and whose rather straight dendrites radiate outward to form glomeruli or to mingle with dendrites of other cells in glomeruli; (2) a peripheral layer of mitral cells and small brush cells presenting a great variety of forms, whose dendrites form the large glomeruli by ramifying in the bundles of fila olfactoria; and (3) of the incoming fila olfactoria and the afferent and efferent fibers connecting the bulb with the rest of the brain.

The gross structure known as the olfactory bulb in the turtle consists of formatio olfactoria and nothing else. While the hippocampal formation and the pyriform lobe both push forward into close contact with the olfactory formation, the groove which marks the peduncle corresponds accurately to the line of division in the internal structure. The so-called nucleus olfactorius anterior in this brain is very clearly distinct from the granule cell layer of the olfactory bulb and does not extend forward beyond the peduncular constriction.

TRACTUS OLFACTORIUS

At the olfactory peduncle the fibers of the olfactory tract (figs. 55, 56, 57) have the following disposition. The largest bundles lie on the lateral surface and pass caudad into the pyriform lobe. These fibers arise from the greater part of the lateral and from the dorsal surface of the bulb. On the dorsal surface of the peduncle a large bundle, which has its origin in the dorsal part of the medial wall, crosses obliquely from the medial to the lateral surface and joins the bundle last mentioned. These constitute the lateral olfactory tract. Its further course will be described in connection with the pyriform lobe. Fibers arising from the remaining ventral and medial parts of the bulb collect into a broad sheet ventral to the large bundle first mentioned. This sheet constitutes the medial olfactory tract. It is thick where it abuts upon the large lateral tract but spreads as a very thin sheet over the ventral and part of the medial surface. This tract runs caudad over the ventro-lateral surface of the tuberculum, gradually diverges from the lateral tract and gives diffuse fibers to the tuberculum and the parolfactory area. The medial tract borders the striatal area in front and below as the lateral tract borders that area dorsally.

Two compact terminal bundles of the medial tract are of especial importance. One bends up into the medial wall between the tuberculum and the commissures and is distributed to this region (gyrus subcallosus) and to the hippocampal formation. This is obviously the equivalent of the olfactory tract component in the precommissural fornix system of mammals. The second bundle continues along the lateral border of the medial tract and runs directly caudad to the amygdaloid prominence, where it enters the nucleus of small cells hereafter to be described as the nucleus of the lateral olfactory tract. This bundle runs along with the large bundle which connects the amygdaloid complex with the hippocampus through the precommissural system (olfactory radiations of Zuckerkandl or fiber bundle of the diagonal band of Broca).

CORPUS STRIATUM

Under this name will be described the structures which are homologous with the chief parts of the corpus striatum in human and mammalian brains. Attention has been called to the fibers of the crus cerebri which are seen in a depression on the lateral surface of the telencephalon. A section at the rostral border of this region (figs. 21, 34) shows two great bundles of fibers cut in cross section, the lateral forebrain bundle or crus and the medial forebrain bundle. The fibers of the crus rise in fascicles (internal capsule) scattered through the outer part of the thick latero-basal wall to the dorsal ventricular ridge and pallial thickening. These include somatic sensory fibers and probably also fibers descending to the motor centers. The medial bundle consists of fibers which connect the olfactory centers with the hypothalamus and lower motor correlation centers. From this bundle fibers rise radially into the dense cell mass forming the floor of the ventricle (figs. 21 to 24, 34).

The whole mass in the latero-basal wall is the corpus striatum. It consists of two portions, a lateral much larger part containing large cells and a medial smaller part containing small cells (figs. 21, 22). The coarser fascicles of the crus rise in a curve through the lateral part into the dorsal ventricular ridge and the general pallium (figs. 32, 33). The finer fascicles of the medial bundle rise in almost straight lines radially in the rostral end of the medial part (fig. 34). In man the condensation of the pallial portion of the crus fibers into a plate-like internal capsule has taken place within a lateral large-celled area, the greater part of which is situated external to the capsule and is known as the lentiform nucleus. The remainder of the large cells together with a dense small celled area next the ventricle constitute the caudate nucleus.

In the turtle the large-celled and small-celled areas are clearly distinct from one another. The small-celled area will be called the caudate nucleus, the large-celled area the lentiform nucleus.

The caudate nucleus presents well marked head and tail portions. Far rostrad (fig. 26) this nucleus forms the floor of the ventricle between the ventral and middle ventricular grooves and occupies almost the entire thickness of the latero-basal wall. At its medial border it is continuous with the lateral parolfactory nucleus. This relation has been described for both reptiles and mammals in a previous paper ('13b, p. 389). Its basal surface is largely covered by the medial forebrain bundle from which fascicles enter the caudate. Followed rostrad in transverse sections the caudate is seen to be covered in by the layers of the olfactory tubercle which are continuous with the parolfactory area medially and the pyriform lobe laterally (fig. 27). In these layers are imbedded the olfactory tract fibers as elsewhere described. Continuing forward, the tuberculum and olfactory nuclei increase in thickness and the caudate grows smaller (fig. 28) until just behind the peduncle the caudate disappears. It is this rostral end of the caudate nucleus that the writer identifies with the nucleus olfactorius anterior of Edinger and other authors. Following sections caudad, the sheet of olfactory fibers and cells scarcely disappear from the surface of the caudate until large cells appear in the lateral wall (fig. 26). Beneath the anterior end of the dorsal ventricular ridge this large-celled nucleus rapidly grows larger and the caudate smaller (figs. 22, 21) until the caudate is confined to a part of the floor of the ventricle adjacent to the ventral groove (fig. 20). From this point the tail of the caudate extends caudally to the point where the middle ventricular groove bifurcates (fig. 15, 10). Before this point is reached the caudate is in connection medially with the anterior nucleus region of the thalamus over the crus and the stria medullaris (figs. 17, 18). The caudate then grows thicker and merges insensibly with the small-celled mass of the amygdaloid complex (nucleus of the lateral olfactory tract) (figs. 15, 16). The caudate then disappears from the sections, its place being taken by an area of neuropile. Horizontal and sagittal sections show in this position a cell-free zone bounding the caudate behind, which corresponds to the ventral arm of the middle ventricular groove as it bends down into the temporal horn of the ventricle.

The caudate nucleus throughout its whole extent consists of small cells without regular arrangement. In its head portion it receives fibers from the olfactory tract and the medial forebrain bundle and gives fibers to the latter. In its tail portion are imbedded the stria terminalis and the great lateral olfactory projection tract of Cajal (figs. 48, 49).

A peculiar vesicular structure found in the head of the caudate and in the lateral parolfactory nucleus should be described here. The greater number of these structures are found around the depression of the ventricle which extends down into the tuberculum but some are found also in the lateral part of the caudate not far from the middle ventricular groove. At first sight these structures appear to be clusters of small cells arranged radially about a core somewhat like clusters of grapes about the stem. The clusters are elongated sometimes in the plane of transverse sections, sometimes obliquely to it. In the latter case they may extend through as many as twelve sections ten microns in thickness. When the clusters are cut either across the long axis or lengthwise of it, the core is almost always seen to contain a lumen around which the cells are arranged (fig. 35). The lumen is bounded by a strong limiting membrane and contains more or less of lightly staining material, resembling mucus. Occasionally a flattened nucleus is seen in the lumen.

In the caudate and parolfactory nuclei these vesicles are found only near the ventricle except in the area where these two nuclei meet beneath the ventral ventricular groove. Here they are found farther from the ventricle, even in the deeper layer of the tuberculum olfactorium. In this region these vesicles lie adjacent to or intermingled with the islands of Calleja, to be described below. It was at first thought, indeed, that these were islands of a special type. The vesicular arrangement and other characters of the cells, however, differentiate these structures sharply from the islands of Calleja. The cells are typically columnar and radially arranged around the lumen, the nuclei usually placed near the peripheral end of the cells. The cell-bodies are often filled with small granules unlike the Nissl-bodies of adjacent nerve cells. The nuclei are small, ovoid and dense, resembling those of ependyma cells rather than those of nerve cells. There are often larger cells, obviously nervous, wedged in among or closely applied to the outer surface of the small cells of the vesicles. Although the fascicles of nerve fibers in the caudate often pass close over the surface of these vesicles and sometimes diffuse fibers appear to run ventrad from a vesicle, I have not found in Cajal or Golgi sections fibers arising from the cells of the vesicles.

I suspect that these vesicles are composed chiefly of ependyma cells and that their lumina represent vestiges of ventricular cavity which have been pinched off during development. This might happen owing to the thickening of the caudate and parolfactory nuclei and consequent encroachment upon the ventral part of the ventricle. The fact that these cell clusters are not penetrated by the neuropile (fig. 35) and the fact that no definite fascicles of nerve fibers are found running to or from them, constitute negative evidence as to their nervous function. At the same time, nerve cells are often found closely related to or imbedded in the ependyma and further study will be necessary to determine the origin, character and significance of these structures.

THE LENTIFORM NUCLEUS

At its rostral end this nucleus appears simply as scattered large cells, outside of the small-celled caudate, among which fascicles rise from the crus to the pallial thickening in the rostral part of the roof. Beneath the rostral end of the dorsal ridge this nucleus has grown to a large body in the lateral wall and has reached the ventricle (figs. 22, 23). From this point caudad a peculiar band of thickened ependyma covers the ventricular surface of this nucleus (figs. 15 to 22, 36). This thick band extends some distance below the middle ventricular groove and bends round that groove into its dorsal wall. While the ependyma elsewhere in the lateral ventricle has from one to three rows of nuclei, this band consists of very tall cells and presents as many as seven to nine rows of nuclei. The peripheral processes of these cells are strong and give a prominent striation to the adjoining nucleus (figs. 36, 37). The curved course taken by these processes which come from the dorsal wall of the middle groove (fig. 37) shows that the groove was formed within the ependymal band and near the border of this nucleus. It also shows how the groove has been formed during the development of the individual by the pushing in of the dorsal ridge which forced this border of the nucleus to be bent over. This thickened ependyma extends right back into the dorsal branch of the middle ventricular groove, where it quickly disappears. Throughout its length this thickened band of ependyma covers a nucleus whose cells are distinctly larger and usually much more numerous than those of the adjoining caudate.

In the striatal area this nucleus makes up the whole thickness of the wall and is traversed by the fascicles of the crus as above noted. Toward the caudal border of the striatal depression (figs. 17, 16) the lentiform nucleus becomes covered externally by a layer of small cells which reaches from the pyriform lobe to the amygdaloid complex and will be described below as the nucleus of the lateral olfactory tract. The lentiform nucleus now decreases in volume caudally, contains several dense collections of cells which are closely related to the thickened ependyma of the middle groove (figs. 16, 33), becomes less rich in cells and finally merges with the central core of the dorsal ventricular ridge behind the end of the middle groove (fig.14).

Throughout its whole length the lentiform nucleus is sharply delimited from the pyriform lobe and (except at its caudal end) from the dorsal ventricular ridge, by cell-free zones. It is marked off from the caudate only by a rather sudden change from large to small cells. The thickened ependyma covering its surface is sometimes separated from that covering the caudate by a slight, sharp groove.

Among the fascicles of the internal capsule which come from the sensory bundle of the crus (see later section on the pallium), are found many cells conspicuously larger than the ordinary cells of the lentiform nucleus. The position of these is indicated in figures 18 and 19. They may prove of importance in further comparisons with the mammalian brain.

TUBERCULUM OLFACTORIUM

The tuberculum consists of a cap of two layers of cells covering the basal surface of the caudate nucleus at its rostral end (fig. 27). The outer layer is sparsely filled with small cells and is closely related at its medial border with the medial parolfactory nucleus and at its lateral border with the pyriform lobe at the sulcus endorhinalis (figs. 26, 27). The inner layer has larger cells and is characterized by the grouping into islands which have been described by Calleja and Cajal. These islands are for the most part clusters of the large cells of the deep layer. They contain from four or five to many cells, which present no regularity of arrangement. The cell bodies are pear-shaped or stellate and are largely filled by vesicular nuclei with prominent nucleoli. In these respects they do not differ materially from the cells scattered between the islands. Some of the cells in the islands take a lighter, more transparent and brilliant stain in neutral red than others.

In addition to the islands of large cells there are a number of dense clusters of small cells which are undoubtedly nerve cells. These clusters do not present a core or lumen and the neuropile does not shrink away leaving a clear space around them as happens in the case of the vesicles described in the caudate nucleus. The true islands are confined to the deeper layer of the tuberculum. It must be noted, however, that the tuberculum extends into the medial wall and merges with the medial parolfactory nucleus. The distribution of the islands may arbitrarily be taken as determining the extent of the tuberculum. The greater number of islands is found near the mediobasal angle and they do not extend far laterally. Caudally the islands disappear from the basal surface where the medial forebrain bundle collects (fig. 24) and are found farther caudad in the medial wall.

There is a very close resemblance between the tuberculum olfactorium in the turtle and the 'superficial basal area' described by the writer in selachians (1911). The writer was unwilling at that time to give the name tuberculum olfactorium to this area because it seemed to include parts of the medial and lateral olfactory nuclei and the region corresponding to the anterior perforate space. The close relation of the tuberculum to medial and lateral olfactory nuclei is common throughout vertebrates and I shall present evidence at another time that the tuberculum and anterior perforate space are by no means wholly independent.

ANTERIOR PERFORATE SPACE

The area corresponding to the anterior perforate space of mammals is not sharply marked off in the turtle. It occupies the caudal part of the basal surface of the large rounded prominence rostral to the optic chiasma. The rostral half of this surface shows the characteristics of the olfactory tubercle. The caudal half lacks these peculiarities and is largely filled by the medial forebrain bundle.

DIAGONAL BAND OF BROCA AND GYRUS SUBCALLOSUS

The large basal mass which includes the head of the caudate, the parolfactory nuclei, the tuberculum olfactorium and the anterior perforate space appears on the medial surface as a rounded area (figs. 3, 6) bounded dorsally and caudally by a groove which descends to the basal surface, bends transversely, and disappears. This groove, as the further description will make clear, is the homologue of the fissura prima of His.

The area dorsal to the horizontal limb of this fissure is the primordium hippocampi (Johnston '13 b). The area between the fissure and the lamina terminalis (fig. 6, *g.s.*) is occupied by a cell mass and a fiber bundle both of which are characteristic of this region in all mammals and perhaps in all vertebrates. In a previous paper ('13 b) this cell mass has been included in the description of the medial nucleus parolfactorius in both the turtle and mammals. This was an error. Since this cell mass lies caudal to the fissura prima in mammals, it is outside the limits of the parolfactory area. It may be spoken of as the *nucleus of the diagonal band.* In mammals this consists of a thin plate of closely packed cells which extends from a point rostral to the anterior commissure down in front of the optic chiasma and caudad on the latero-basal surface to the amygdaloid complex. It is very sharply marked in the rabbit and has been figured in the opossum and bat ('13 b, figs. 28, 42). The distinction between this nucleus and the medial parolfactory nucleus is shown in figure 46 of the paper referred to, where this nucleus consists of small cells rostral to the anterior commissure and is not separately labelled. The cell-free zone between this and the medial parolfactory nucleus corresponds to the fissura prima.

In the turtle the same plate of cells is found (figs. 20, 21) occupying the medial surface rostral to the anterior commissure and extending down close in front of the preoptic recess, near the large-celled supraoptic nucleus (fig. 19), and continuing laterally and caudally ectal to the medial forebrain bundle (fig. 18) until it expands into the large anterior nucleus of the amygdaloid complex from which arises the great olfactory projection tract (fig. 17). The relations to the amygdaloid will be further described in the next section. The extreme rostral and upper end of this nucleus is the very dense collection of cells seen near the recessus superior in figures 19 and 39. There is no break in the continuity of the nucleus described but on the basal surface in front of the optic chiasma and lateral to the optic tract, the number of cells is much less than in the medial wall. Throughout the length of the nucleus its cells are small and are somewhat elongated in the direction of the densely packed bundle of fine fibers among which they lie.

The fibers in question enter into the precommissural bundles of the fornix system in the medial wall. They are the olfactory radiations of Zuckerkandl and constitute the chief pathway from the amygdaloid region to the hippocampus. Compare '13 b, p. 410. From the amygdaloid forward along the basal surface these fibers form a low ridge parallel with the optic tract (figs. 1, 8, 19) and in this position the bundle is clearly visible in the entire brain (fig. 2). The bundle is clearly distinct from the medial forebrain bundle which lies ental to this. In front of the optic chiasma the bundle is seen to be composed of two parts, one of which is derived from the medial olfactory tract as above described, while the other bends up into the medial wall, spreads out in the area between the fissura prima and the commissures, and enters the hippocampus (figs. 56, 57). The triangular area between the fissura prima and the commissures in which this bundle spreads out like a fan is the equivalent of the mammalian gyrus subcallosus. Lateral to the optic chiasma this bundle passes close to the supra-optic nucleus and there seems to be a connection between the two which has not yet been carefully studied.

The homology of the area described in this section with the gyrus subcallosus and the diagonal band of the mammalian brain is obvious. Its chief morphological significance is that it represents the persistence of the preoptic and precommissural connection between the medial olfactory area and hippocampus on the one hand and the lateral olfactory area and amygdaloid complex on the other hand. In the ichthyopsid brain where the temporal pole has not yet been formed, the continuity of the corresponding regions is broad and direct. The size and functional importance of this connection in reptiles and mammals is of great significance for the explanation of the evolution of the temporal pole and the general pallium.

LOBUS PYRIFORMIS

As already indicated the pyriform lobe forms a somewhat prominent ridge along the dorso-lateral surface of the hemisphere, extending from the olfactory peduncle into the caudal pole. It is separated from the general pallium dorsally by the fissura rhinalis. Rostrad this fissure runs obliquely mesad and joins the peduncular groove or constriction as it bends down into the medial wall. Caudally the fissura rhinalis presents considerable individual variation in its depth and length. Usually it fades away at about the middle of the hemisphere (fig. 1) so that there is no gross boundary line between the caudal part of the pyriform lobe and the general pallium. In the rostral part of the hemisphere a slight sulcus endorhinalis approximately marks the boundary between the pyriform lobe and the area occupied by the tuberculum and corpus striatum (figs. 22 to 26). The caudal continuation of this sulcus becomes much deeper between the pyriform lobe and the striatal area (fig. 1) and bends down between the striatal area and the amygdaloid prominence. At the point of bending is the deepest part of this groove and from this point the amygdaloid fissure extends caudad as above described.

The pyriform lobe consists of the lateral olfactory tract and the special gray matter accompanying the tract. Sections through the middle or rostral part of the lobe (figs. 19, 25) show that it consists of a superficial fiber layer and a deeper plate of cells and that it is everywhere very clearly and sharply separated from the border of the pallium, the dorsal ventricular ridge and the lenticular nucleus by a cell-free zone. This cell-free zone is more sharply marked than any other in the brain.

The outer surface of the lobe is covered by a continuous sheet of fibers of the lateral olfactory tract. These arise in larger part from the lateral wall of the olfactory bulb and in smaller part from the dorsal and medial wall. The former fibers enter the extreme rostral end of the pyriform lobe on the lateral surface of the peduncle; the latter cross from the medial to the lateral side in the peduncular groove on the dorsal surface and join the former fibers to make up the common tract (figs. 55, 56).

Just behind the peduncular constriction the pyriform lobe appears to thicken rapidly. This thickening forms a conspicuous rounded prominence on the dorso-lateral surface of the hemisphere (figs. 26, 5). The arrangement and relations of the cell masses in this prominence show at once that it is made up of two parts. The surface layer of cells belongs to the pyriform lobe and is no thicker here than elsewhere. The elevation here is due to the deep layer of cells which is the thickened lateral border of the general pallium (figs. 24, 25, 26). This is indeed a lateral extension of the dorsal pallial thickening, which lies beneath the pyriform lobe as the lateral border of the pallium does throughout the rostral two-thirds of the lobe. The pyriform lobe, then, is not actually thickened but is merely bulged out by the thickening of a pallial formation beneath.

The majority of cells in the pyriform lobe are large multipolar cells with large dendrites. In regard to this a comparison of the pyriform lobe with other parts of the forebrain reminds one of the comparison between Deiters' nucleus and adjacent nuclei in the medulla oblongata. The cells are noticeably larger in the rostral half of the lobe than in the caudal, the largest cells and the greatest proportion of large cells being found just behind the prominence above mentioned. In the caudal part the cells become distinctly smaller and where the lobe broadens out to form the lateral wall of the ventricle, the cells take on pyramidal forms much like the cells of the general pallium or the hippocampus.

Throughout the rostral half of the pyriform lobe small cells are almost wholly confined to its ventral portion (figs. 19, 25, 40). These small cells extend somewhat below the sulcus endorhinalis, and the cell-free zone, which accurately marks the boundary of the lobe, meets the surface ventral to the sulcus endorhinalis. This sulcus is therefore actually within the area of the pyriform lobe. At the caudal border of the striatal area the band of small cells along the ventral border of the pyriform lobe spreads ventrad in a broad sheet across the base of the amygdaloid fissure (fig. 5) and expands into a large nucleus of small cells occupying the lateral and rostral part of the amygdaloid prominence (figs. 17, 16). This sheet of small cells is accompanied by a large bundle of fibers from the lateral olfactory tract which ends in this nucleus. This is the nucleus of the lateral olfactory tract (figs. 5, 17).

A further examination of the relations of the small cells in the pyriform lobe shows that essentially two nuclei or two cell groups are to be distinguished. The small cells in the region of the amygdaloid fissure are very clearly separated from the large-celled part of the lobe by a space or cell-free zone (figs. 15, 16, 17). Farther forward although there is no space separating them, the small cells do not mingle with the large cells and the small-celled band becomes continuous with the superficial layer of the tuberculum and through that with the medial olfactory nucleus (fig. 26). The large-celled nucleus, on the other hand, maintains its identity rostrad to the peduncle as already described (fig. 28). Moreover, the location of the small cells below the endorhinal sulcus gains significance from the fact that at the level of the tuberculum the small-celled band spreads ventrally to become continuous with the superficial layer of the tuberculum. The cell-free zone which limits the pyriform lobe internally now becomes continuous with the limiting zone which appears between the caudate sulcus and the layers of the tuberculum (figs. 26, 27).

These relations when summed up with the description that has gone before show that there is a continuous area of small-celled olfactory nuclei comprising the medial parolfactory nucleus, the superficial layer of the tuberculum, the small-celled band of the pyriform lobe, the small-celled nucleus of the amygdaloid complex and the nucleus of the diagonal band. These together constitute a continuous ring which surrounds the crucial-striatal area.

The whole of this ring receives fibers from either the lateral or medial olfactory tract. This continuous complex of small-celled nuclei seems to serve as an intermediate selecting and distributing station through which olfactory impulses are forwarded to the cortical center for sensation, the hippocampus; or to one of the motor correlation centers in the diencephalon, or to centers lower down the brain stem (compare Johnston '15 a). The greater part of the pyriform lobe made up of large cells is probably concerned with olfacto-somatic correlation. Other places for correlation of olfactory and somatic impressions are probably found in the amygdaloid complex and in the hippocampus (subiculum). The distribution of large cells in the pyriform lobe probably gains significance from their relation to the pallial thickening.

The boundary line between the pyriform lobe and the general pallium is less distinct in the caudal part than elsewhere. As the pyriform lobe is followed caudad in sections to the point where it forms the whole thickness of a part of the wall of the ventricle (figs. 13 to 15) the cell-free zone at first continues as a very clear oblique dividing line in this wall (fig. 14). This overlapping of the general pallium by the border of the pyriform lobe is very characteristic. This dorsal border of the lobe rises dorsally in its caudal portion as the model clearly shows (fig. 5). In transverse sections through the region in which the small-celled portion of the pyriform lobe spreads ventrad to merge with the nucleus of the lateral olfactory tract, the cell masses of the dorsal ventricular ridge begin to be connected by a thick curved layer of cells with what appears to be the cell layer of the pyriform lobe in the lateral wall (fig. 15). When this relation is studied in sections farther caudad (figs. 11, 12) it is clearly seen that it is the general pallium which thus enters into continuity with the ventricular ridge. In transverse sections there is no sharp boundary between pyriform lobe and general pallium. It is noticed, however, that there is a narrow portion of the wall adjacent to the dorsal boundary of the lobe in which the cells are placed close to the ventricular surface (fig. 12). The oblique line bounding the lobe dorsally at length gives way to a narrow area in which the cells of the lobe meet those of the pallium above in a confused mass. The ventro-caudal margin is not clear. Sections give the impression that the pyriform lobe merges insensibly with the general pallium in this region. In the model this boundary has been fixed arbitrarily.

THE AMYGDALOID COMPLEX

When the hemisphere of the turtle brain is viewed from the lateral and basal aspects (figs. 1, 2, 5, 6) the region which is occupied in part by the amygdaloid complex appears as a basal prominence similar to the temporal pole of the mammalian brain. It is bounded rostrally by the crucial-striatal area, medially by the optic tract and thalamus and laterally by the amygdaloid fissure. Caudally this prominence passes insensibly into the rounded caudal pole.

Nucleus of the lateral olfactory tract. As above described, a part of the lateral olfactory tract bends down across the amygdaloid fissure just caudal to the striatal area and ends in the lateral border and rostral tip of the amygdaloid prominence (fig. 8). This region is therefore clearly homologous with the nucleus of the lateral olfactory tract in the mammalian brain. This nucleus is composed of small cells which occupy the tip and lateral part of the amygdaloid prominence and extend in a thin layer across the amygdaloid fissure to meet in a thickened border the large-celled nucleus of the pyriform lobe (figs. 16, 17). The two nuclei are separated by a cell-free zone and this condition continues back to the end of the nucleus (fig. 13). At the caudal border of the striatal area the thin layer of small cells is traversed by the fibers of the lateral olfactory tract which bend down into this nucleus, and by numerous fibers from the pyriform lobe which pass over the surface of the nucleus to join the stria medullaris (fig. 56). From the tip of the amygdaloid prominence the fibers of the diagonal band go forward as above described. These are also mingled with the stria medullaris. At the same time the small cells of the diagonal band merge with the small-celled nucleus of the lateral olfactory tract (fig. 17).

Where the thin layer of small cells forms the boundary of the striatal area it covers in the large-celled lateral nucleus of the corpus striatum (nucleus lentiformis) (fig. 16). In transverse sections the nucleus lentiformis is followed caudad for some distance beyond this, always traversed by bundles of crucial fibers which are bending up into the pallium and the dorsal ventricular ridge. The nucleus lentiformis gradually grows smaller and is replaced by the small-celled nucleus which grows thicker caudally and enters into close relations with the caudate nucleus. Indeed, in its rostral portion the entire amygdaloid eminence is filled with small cells, the ventricular portion being the caudate nucleus, the superficial portion being the enlarged continuation of the nucleus of the lateral olfactory tract (fig. 17).

Relations of the caudate nucleus. It has been stated that the caudate ends about opposite the end of the middle ventricular groove and is bounded by the branch of that groove which bends down into the temporal horn of the ventricle. Here the caudate merges with the nucleus of the lateral olfactory tract in a small-celled mass which constitutes the chief part of the amygdaloid complex. In the lizards this mass, or the caudate part of it, is greatly enlarged and forms a prominent lobe or ridge in the ventricle. C. L. Herrick showed that this was an important olfactory center and it is this lobe which was first called epistriatum by Edinger. It was this body whose relations very naturally suggested to Kappers ('08) that the epistriatum of lower vertebrates formed or entered into the amygdaloid complex of mammals. In the turtle this body is simply a part of the amygdaloid complex without hypertrophy. However, if all the other structures to which the term epistriatum has been applied were to be gathered into it, the amygdaloid complex would be a veritable museum!

The caudate nucleus at its rostral end is closely related to the olfactory tubercle and parolfactory area and receives fibers from the medial olfactory tract. At its caudal end it enters into close relations with the nucleus of the lateral olfactory tract and helps to form the olfactory part of the amygdaloid complex.

Medial large-celled nucleus. In sections through the rostral part of the amygdaloid complex the small-celled mass is covered basally by a rounded cap consisting almost wholly of neuropile (figs. 16, 42), only a few medium sized cells being imbedded in it. This neuropile is formed by the interlacing stria medullaris, lateral olfactory tract and olfactory radiations as above described. As the sections are followed caudally the cells in this neuropile increase in number and size until a conspicuous nucleus forms the basal portion of the complex (figs. 15, 43). This is the condition in sections which pass through the caudal border of the stria medullaris. Just caudal to this (figs. 14, 44) the temporal horn of the ventricle penetrates this nucleus. Farther caudally the large cells are all found in the lateral or amygdaloid wall of the ventricle while the hippocampal formation and fimbria appear in the medial wall. The large-celled nucleus lies ventral to the tail of the caudate nucleus. This is the region in which the tail of the caudate merges with the small-celled nucleus of the lateral olfactory tract. Following the sections caudad, the caudate rapidly diminishes and the large-celled nucleus spreads farther dorsad in the large ventricular ridge (figs. 13, 45). At the same time the nucleus of the lateral olfactory tract grows smaller and appears as a rather dense mass of cells near the amygdaloid fissure. Now there appears in the ventral wall between this and the hippocampus an area of scattered cells which on being followed further caudad is found to belong to the general pallium (which see). By the time this condition is reached the caudate has entirely disappeared from the sections and the large-celled nucleus has spread dorsad to the level of the dorsal branch of the middle ventricular groove. Here this nucleus constitutes a ventricular layer of large cells rather closely packed but not arranged in clusters as are the cells of the dorsal part of the dorsal ventricular ridge. Further from the ventricle the lower part of the ridge is filled by the same irregularly placed cells as are found in the dorsal part. Both these and the ventricular layer of large cells merge with the general pallium in the ventral wall. The ventricular layer representing the large-celled nucleus continues over the medial surface of the basal lobe of the ridge to its caudal end.

Further evidence as to the individuality of this nucleus is found in its fiber connections. Only the general disposition of its chief tract will be described here. In the extreme tip of the temporal horn of the ventricle (figs. 10, 13, 14) is seen a small ridge which follows the ventro-rostral border of this nucleus. It is clearly seen in the model and in sections in all planes. This ridge is composed chiefly of fibers which run caudad over the medial surface of this nucleus. In addition to the bundle which forms the ridge there is a thin sheet of fibers in small fascicles covering the whole medial face of this nucleus. All of these fibers gradually disappear as they are traced caudad over this nucleus, in which they have either their origin or ending. Traced rostrad all these fibers pass through the tail of the caudate as a thick bundle. Here the bundle is just lateral (internal) to the stria medullaris as that bundle rises from the nucleus of the lateral olfactory tract to enter the thalamus. The bundle here described now forms the most compact part of the stria terminalis and in addition a large part of its fibers go forward diffusely in the substance of the caudate nucleus. A further discussion of the relations of this bundle will be reserved for a later study of the stria terminalis system. It is evident, however, from the facts given, that what is here described as the medial large-celled nucleus belongs to the amygdaloid complex and the distribution of the stria terminalis bundle confirms the observations as to its extent, based upon the character and grouping of cells.

Dorsal ventricular ridge. Farther caudally in transverse sections the ventricle pushes around both laterally and ventrally until the whole ventricular ridge is seen attached to a pallial wall and no longer to the brain stem (figs. 11, 12). In this pallial wall the extent of the general pallium and hippocampal formation can be made out, at least roughly, as represented in the model. The dense, deep layer of cells of the pallium turns into the ventricular ridge where it is continued by layers of cells lying adjacent to the ventricle. The sparsely scattered cells in the outer part of the pallial wall are continued into the core of the ventricular ridge where they are somewhat more compactly arranged than in the pallium. The section passes through the caudal part of the amygdaloid fissure. It is readily seen that the ventricular ridge corresponds to the external groove and that the fissure is a true fold of the brain wall. Examination of the relations of all parts of the dorsal ridge gives the impression that the ridge is an infolding of the brain wall from this region and that it has pushed forward well toward the rostral end of the hemisphere. Its cell layers come into continuity with those of the pallium here in the amygdaloid fissure and with no other nucleus whatever. They are sharply distinguished from those of the caudate and lenticular nuclei and from the pyriform lobe and are usually separated from these by cell-free zones (figs. 11 to 22). In its rostral part the ridge is sharply separated from all other cell masses and projects freely into the ventricle.

In a transverse section taken at the level of the stria medullaris the cells in this ridge present the arrangement seen in figure 16. All around the ventricular surface are a great number of large and small clusters of cells. Some of these clusters are large, rounded and have a definite outline. Others are very irregular and their outlying cells mingle with the scattered cells between the clusters. The individual cells of the clusters do not differ materially in size from those of the adjacent general pallium but are conspicuously larger than those in any other part of the same section. In the lower part of the ridge, adjacent to the middle ventricular groove, there is a tendency for the cells to be closely aggregated in a large area without special clusters.

The core or central part of the ridge is largely filled by fibers among which are scattered cells. These cells are smaller than those of the clusters. The ventral part of this area is encroached upon by the lentiform nucleus and by the main body of fibers entering or leaving the ridge (figs. 15 to 22). The dorsal part of the central area of the ridge contains a larger number of cells almost evenly scattered. Many of these cells approach in size those of the clusters. This part has almost the appearance of an independent cell-mass and for convenience may be called the core-nucleus. It extends through nearly the whole length of the ridge as a large, somewhat quadrilateral area bounded laterally by the pyriform lobe and centrally by the cell clusters of the ventricular layer Dorsally it is separated from the thickened border of the general pallium by a cell-free zone of fibers and ventrally it is bounded by the chief fiber mass in the center of the ridge. Toward the rostral end of the ridge this body of cells becomes separated into two masses in the transverse section, owing to the general pallium and lentiform nucleus pressing into it from above and below respectively (fig. 22). Caudally this mass of cells becomes less distinct and merges with the superficial cells of the general cortex as noted at first. Throughout the greater part of its length this core-nucleus is penetrated by numerous fibers which pass up from the internal capsule to the thickened lateral border of the general pallium. Although only a few such fibers are seen in Weigert sections, a great number of non-medullated fibers take this course and they are very conspicuous in Cajal preparations.

Comparison of the turtle brain with that of some lower mammals leads to the suggestion that the dorsal ventricular ridge is the homologue of one of the cell masses which constitute the amygdaloid complex in mammals. This is entirely independent of the homology of the 'epistriatum' with the amygdaloid body suggested by Kappers and others. This ventricular ridge is a wholly different structure from the 'epistriatum' of Kappers. This matter will be discussed in a later paper dealing with the amygdaloid complex in the opossum and some mammals. To follow the comparison here would lead beyond the limits of this paper.

The amygdaloid complex in the turtle includes the following structures: the nucleus of the lateral olfactory tract, the tail of the caudate nucleus, the medial large-celled nucleus and probably the dorsal ventricular ridge. The amygdaloid eminence in the temporal pole is encroached upon by both the hippocampus and the general pallium. The amygdaloid complex has related to it the lateral olfactory tract, a bundle of the medial olfactory tract, the bundle of the diagonal band, the stria medullaris, the stria terminalis, fibers of the sensory radiations from the thalamus, an olfacto-hypothalamic projection tract, and an anterior commissure bundle.

The simple enumeration of these tracts shows that while the amygdaloid complex in mammals is a compact collection of cell masses occupying a restricted area in the temporal pole, it is a complex of very diverse elements which have been brought together by mechanical forces and have no primary functional unity.

The extent of the hippocampal formation. The morphological relations of the hippocampal formation have been treated with some fulness in a previous paper ('13 b). There is now to be noticed the boundary between hippocampus and general pallium and the relation of the hippocampal formation to the amygdaloid complex. In the rostral part of the hemisphere the characters shown in figure 47 enable us to fix the boundary of the hippocampus with some degree of accuracy on the basis of cell arrangement. Above the fimbrio-dentate sulcus, which separates the homologue of the fascia dentata from the primordium hippocampi and fimbria, there are first pyramidal cells compactly arranged in a ventricular layer with few peripheral cells. The cells are smaller than the cells in most parts of the general pallium but are neither so small nor so closely crowded as the cells in the deep layers in the middle part of the dorsal pallium in this region. This is the portion which is now generally accepted as the homologue of the fascia dentata of mammals. There is no clear separation or distinction between fascia dentata and hippocampus in this turtle's brain.

Dorsal to this, the cells in the medial wall are less closely arranged, appear larger and are less deeply stained. Many cells here are placed far from the ventricle, even lying in the outer half of the wall. Many cells adjacent to the ventricle are distinctly pyramidal in form and have large, dominating apical dendrites. This loose arrangement of cells rather suddenly gives way to a compact arrangement near the dorso-medial angle of the hemisphere. The dorsal area of compact cells will be described as part of the general pallium. The area of loose cells probably belongs to the hippocampal formation and the transitional region called the subiculum.

As transverse sections are followed caudad the area which is here assigned to hippocampal formation and subiculum grows rapidly wider. There is nowhere a sharp line of demarcation between hippocampus and general pallium, and none is to be expected. There is everywhere noticeable the point at which the deep cell layer becomes abruptly more compact. This point has been taken as the medial boundary of the general pallium in making the model so that what is there shown as hippocampal formation probably includes the equivalent of the subiculum as well (see fig. 6).

As the sections are traced caudad there is a gradual change in the size of the cells. The cells of the hippocampal formation become smaller, those of the dorsal general pallium somewhat larger, until the difference is at first noticeable, then conspicuous. From the level of the commissures caudad the cells of the hippocampus are much smaller than those of the general cortex.

In the occipital and temporal poles of the hemisphere essentially the same characters are found. Near the fimbria border is an area of dense cells in a deep layer. Next to it is an area with cells loosely arranged and many of them near the periphery (fig. 45). In the temporal region this area of loose cells is narrow and is accompanied by an internal sulcus which serves as an additional landmark. The boundary shown in the model made from transverse sections has been carefully checked up by means of horizontal sections and found to be essentially correct.

The fimbria passes over the interventricular foramen and continues back along the border of the hippocampal formation to which the choroid plexus is attached (fig. 57). The bundle decreases in size as it proceeds but is still fairly large at the caudal end of the choroid fissure. Here the fimbria bends around the end of the fissure and spreads in the medial wall of the ventricle medial to the position of the large-celled amygdaloid nucleus. This is sufficient evidence that the hippocampal formation extends around the end of the choroid fissure into the medial wall of the temporal pole and confirms the conclusion based on the study of the cells.

In the temporal pole the hippocampus comes into contact with the general pallium and the large-celled amygdaloid nucleus only, not at all with the nucleus of the lateral olfactory tract or any of the small-celled portion of the amygdaloid complex (fig. 8). The hippocampal formation is intimately related in front with the olfactory bulb and secondary olfactory centers. Caudally it forms the border of the pallium adjacent to the choroid fissure and is bounded on the other side by general pallium. There is no continuity of cell masses between the caudal end of the hippocampus and the caudal part of the secondary olfactory centers. The olfactory apparatus taken as a whole does not form a complete ring following the line of junction of the hemisphere with the brain stem ('limbic lobe'). An opening in this supposed ring occurs between the nucleus of the lateral olfactory tract and the hippocampus.

THE GENERAL PALLIUM

The general pallium occupies the dorsal and caudal walls of the hemisphere and reaches well into the temporal pole. Throughout its whole extent it is interposed between the hippocampus on the one hand and the pyriform lobe and nucleus of the lateral olfactory tract on the other. The pyriform lobe and hippocampus meet one another in front of the rostral end of the general pallium near the olfactory peduncle (fig. 7). The general pallium extends forward slightly further than is shown in the model. The presence of a subiculum between the hippocampus and general pallium and of a definite boundary line between the dorsal border of the pyriform lobe and the general pallium has been described above.

Only the most general features in the structure of the pallium can be described in this paper. By far the greater part of the cell bodies are collected in a dense layer near the ventricle. At the dorso-medial border the cells are mostly pyramidal and there are a few cells scattered in the outer part of the pallium. There are many more of the peripheral cells in the adjacent subiculum.

Farther laterally the layer is more compact and the pyramidal form of the cells less evident.

The lateral border of the pallium presents marked characteristics in the rostral half of the hemisphere. In transverse sections the cell layer has a curve near the lateral border which is slight in the caudal part of the hemisphere (fig. 15), becomes more marked rostrally (fig. 17), and near the rostral end is strongly S-shaped (figs. 18, 19). This, together with the slightly larger size of the cells and their arrangement in a very compact layer, suggests rapid growth and mechanical crumpling of the cell layer. This is the border along which internal capsule fibers enter and leave the pallium and there is doubtless a difference of function between this border and the dorsal or medio-dorsal parts of the general pallium. This lateral border becomes gradually thicker as it is followed rostrad and dips down and becomes more and more overlapped by the pyriform lobe. Here the compact plate of cells becomes a thicker mass which in some sections is broken into several masses (figs. 23, 24). At the rostral end this thickened lateral border produces the pallial thickening mentioned in an earlier section.

Toward the rostral end of the pallium the cell layer becomes much less compact except in the lateral thickened border. The pyramidal cells with prominent apical dendrites are mostly confined to the deep layer and outside of these are many cells of stellate or ovoid form. These cells are present in the whole width of the pallium including the subiculum and the lateral thickening. In some sections these cells seem to constitute an incompletely separate layer. When the pallial thickening begins to form a ridge projecting into the ventricle it is noticed that these cells are more numerous in this part of the pallium than elsewhere (figs. 21, 22) and from this point on rostrad they form a conspicuous outer layer of cells in the pallial thickening (figs. 23, 24, 25, 40).

Near its rostral end the total number of cells in the pallium is greatly increased, the deep layer is much thickened and very dense and the outer layer extends well toward the medial border. Indeed, nearly the whole width of the pallium at its rostral end bears the characteristics of the lateral thick border. In other words, it may be stated that the body recognized in the gross brain as a pallial thickening occupies the rostral end of the general pallial area and extends caudad as a gradually diminishing lateral thick border. The pallial thickening is characterized by the presence of a well-developed peripheral layer of cells and this suggests a special functional significance for this body.

The pallial thickening last described is the most highly specialized part of the general pallium and is farthest removed from the brain stem. The temporal portion of the pallium, adjacent to the amygdaloid fissure, is the most primitive and simple in structure. In the caudal part of the basal surface of the hemisphere, where the temporal and occipital poles may be said to meet, the general pallium presents a somewhat more open arrangement than in most of the pallium (fig. 46). A larger number of cells are placed near the outer surface. From this point rostrad, toward the amygdaloid eminence, there is the line of transition between pallium and hippocampus and an area of pallium in which the cells become more and more irregularly scattered as one follows the sections forward (figs. 45, 44). At its lateral border this pallial area bends inward to become continuous with the cell masses in the dorsal ventricular ridge (figs. 11, 12). As the sections are followed forward it becomes evident that this part of the pallium is related to that basal and caudal part of the ventricular ridge whose ventricular surface is covered by the large-celled nucleus of the amygdaloid complex (fig. 44).

In sections which pass through the caudal part of the pyriform lobe and the deeper and broader part of the amygdaloid fissure (fig. 13), the caudo-lateral prolongation of the nucleus of the lateral olfactory tract appears in the depth of the fissure. Now the general pallial area on the basal surface is bounded by the hippocampus medially and by the nucleus of the lateral olfactory tract laterally. Internally the pallial cells merge with those of the basal lobe of the ventricular ridge. As the sections are followed forward the nucleus of the lateral olfactory tract increases in size while the hippocampal formation disappears from the sections before the tip of the temporal horn of the ventricle is reached (figs. 45, 44). At the same time the tail of the caudate nucleus appears between the dorsal and basal lobes of the ventricular ridge. The large-celled nucleus now surrounds the temporal horn of the ventricle and occupies the pallial surface medial to the nucleus of the lateral olfactory tract. Now the large bundle of the stria terminalis goes upward and forward from the large-celled nucleus, as above described. Finally the large-celled nucleus disappears from the sections at the level of the stria medullaris.

Thus the general pallium extends forward in the basal wall to the tip of the temporal pole. Its cells in this region are of various forms and are irregularly scattered and there is an intimate merging of the pallium with the large-celled nucleus of the amygdaloid complex. There is no area or avenue of continuity of the cell masses of the pallium with those of the brain stem. There is, however, continuity of the pallium, the large-celled amygdaloid nucleus and the lentiform nucleus of the corpus striatum. The lentiform nucleus imbeds the lateral forebrain bundle and is therefore comparable with the somatic area in the lateral wall of the selachian forebrain. In the turtle there is continuity throughout the cell masses related to the afferent and efferent fibers primarily somatic in function in the lateral bundle, or crus. In the fishes the somatic area is continuous with the sensory centers of the thalamus. That this connection has been lost in the turtle may be attributed to the greater development of the hemisphere which has elongated the crus and drawn the lentiform nucleus away from the thalamus.

The general pallial portion of the hemisphere consists therefore of (1) a broad, rather thin wall forming the roof and caudal pole of the hemisphere; (2) bounding this laterally and anteriorly, a pallial thickening which has a peculiar structure; and (3) a temporal area of simpler structure which is intimately connected with the dorsal ventricular ridge. This ventricular ridge might, indeed, logically be listed as a fourth part of the general pallium.

That these four regions are intimately related and constitute one complex area in the forebrain, comparable to the olfactory area with its several nuclei, is attested at once by the distribution of the fibers of the cerebral peduncle or internal capsule. This great bundle, where it is about to pass from the brain stem to the hemisphere (fig. 33), is bounded laterally by the optic tract and dorsally by the stria medullaris and stria terminalis. Medial and medio-ventral to this bundle is a large area of fibers which contains (1) the olfactory projection tract of Cajal ('04, fig. 778) descending from the olfactory portion of the amygdaloid complex, and (2) the medial forebrain bundle which follows the crus into the hemisphere and runs forward in relation to the tuberculum, caudate and other olfactory nuclei.

The great lateral forebrain bundle or crus is composed of two parts, dorsal and ventral. The dorsal part is coarse-fibered and well medullated. It comes from the nucleus rotundus and adjacent cell masses in the dorsal part of the thalamus which correspond to the thalamic sensory nuclei in mammals. The ventral part consists of finer fibers which are well medullated at least in their course through the thalamus and corpus striatum. This bundle bends somewhat ventrad and runs caudad on the ventro-lateral surface of the thalamus and mid-brain until some distance behind the third nerve where it becomes more diffuse and seems to end at least in large part before the isthmus is reached. Without going further into descriptive details it is evident that the dorsal bundle contains the sensory radiations from the thalamus to the hemisphere and that the ventral bundle corresponds at least roughly to the efferent tracts from the hemisphere as we know them in mammals.

These two bundles behave differently in the hemisphere. The dorsal deeper bundle bends up rapidly in larger and smaller fascicles through the lateral part of the striatum and enters the core of the dorsal ventricular ridge. The fascicles spread rather wide apart as they pass through the striatum but are all deep beneath the surface of the brain. Upon reaching the ventricular ridge the fibers enter into the formation of a uniform lace-work in the meshes of which the cells of the core-nucleus are evenly scattered. From this lace-work many fibers proceed into the dorsal pallium. This is the appearance seen in Cajal preparations. In Weigert sections the fibers appear to lose their myelin as they enter the dorsal ridge but a small number of medullated fibers are seen running right through the ridge into the pallium (fig. 32). There is at present insufficient evidence to show what proportion of the non-medullated fibers of the sensory bundle reach the pallium but it is clear that a large part of them do so. When transverse sections are followed from behind forward it is seen that the coarse-fibered sensory bundle all rises to the pallium or the ventricular ridge and disappears from the sections before the rostral end of the ridge is reached. The distribution of fibers to the caudal pole of the pallium is partly through the base of the ventricular ridge and partly by way of the fiber-layer of the pallium itself. Very few medullated fibers reach the caudal pole.

The ventral, fine-fibered bundle spreads laterally in the superficial part of the striatum (nucleus lentiformis) and rises toward the lateral border of the pallium. The fascicles, however, slant forward more as they run dorsally and the whole bundle extends much farther forward than the deeper coarse-fibered bundle. The fascicles break up into diffuse fibers before the pallium is reached and many of the fibers may arise from the lentiform nucleus itself. It is clear, however, that the greater part of these fibers enter into the fiber-layer in which the lateral thickened border of the pallial cell-layer is imbedded. Moreover, the greater part of this bundle is related to the pallial thickening.

In sagittal sections (fig. 51) fascicles from the superficial bundle are traced directly up to the pallial thickening through the rostral part of the striatum, and also around the rostral end of the striatum through the cell-free zone between the striatum and the pyriform lobe. It is interesting to note in sagittal sections that medullated fibers enter the pallium and the ventricular ridge only in the rostral part (half or more) of each. The bundles which rise from the crus into the core of the ventricular ridge lose their myelin or disperse so that they can not be traced farther as fascicles.

In Chrysemys marginata the ventricular ridge is smaller than in Cistudo and does not extend as far forward. The pallila thickening on the other hand is relatively more extensive. In this form the direct course of the superficial fibers of the crus up to the pallial thickening is much more clear than in Cistudo.

It is hoped to test the disposition of these fibers experimentally. If the above conclusions drawn from the study of sections prove to be correct, it is evident that the pallial thickening must be regarded as the chief source of the fibers descending from the pallium to the brain stem. It should be noted that the pallial thickening occupies the same position in the hemisphere as that occupied by the motor area in the lower mammals, except that there is no appreciable frontal association area seen in front of it. Also, the descending fibers lie in front of the sensory radiations as they do in the mammalian internal capsule. It seems probable that the pallial thickening is chiefly motor (efferent) in function and that the ventricular ridge and a large part of the general pallium are sensory and associational. However, it is probable from my present results that the sensory and motor areas are not wholly independent. Also the whole subject of localization within the sensory field is untouched in the present paper.

SUMMARY AND COMMENTS

The advantage offered by the turtle brain for the study of the evolution of the mammalian cerebral hemisphere is that the turtle occupies such an intermediate position between fishes and mammals that it is possible from the standpoint of the turtle brain to recognize the homologous structures in all three groups and to explain the changes which have taken place from the brain of the fish to that of the turtle and from the brain of the turtle to that of the marsupials and mammals. This is due to the fact that among living reptiles the turtles are most nearly related to the ancestors of mammals and still possess a relatively simple and slightly specialized brain. In some points there would be great advantage from the study of the dipnoan brain, to bridge the gap between the fishes and reptiles. This gap is much wider than that between the reptiles and mammals, since neither the specialized fishes nor the amphibians stand in the line of descent of reptiles and mammals. The dipnoans, or some of them, do stand in this line of descent and the evolution of the brain must be traced from the selachians, through the dipnoans, reptiles and marsupials to the mammals.

The main purpose of the present paper is merely to define the morphological relations of the chief cell masses in the brain of the turtle in order that these may be used in future studies as points of reference in comparing ichthyopsid, reptilian and mammalian brains. This is done in the belief that a satisfactory account of the mammalian and human brain must eventually include an account of the origin and evolution of the several structures together with an explanation of existing structural relationships and the course of their evolution on the basis of environment, habits of life and the function of the several parts.

The olfactory area

Upon comparing the secondary olfactory centers of the hinge turtle with those of fishes, the only very noteworthy fact is the appearance in the turtle of a nucleus of the lateral olfactory tract behind the area occupied by the lateral forebrain bundle. This is a distinctly mammalian character. In the selachian (Johnston '11 a) the lateral forebrain bundle is imbedded in the somatic area in the lateral wall of the slender portion of the forebrain known to many authors until recently as the 'praethalamus,' and the lateral olfactory area lies wholly in front of this. The relations are shown in figure 59, in which are roughly indicated the areas from which alone the pyriform lobe and the nucleus of the lateral olfactory tract may have been developed. In the turtle these areas have moved far backward on the lateral surface and the mass which imbeds the lateral forebrain bundle appears as an island surrounded by olfactory nuclei,—the striatal area surrounded by the pyriform lobe, nucleus of the lateral olfactory tract, the diagonal band, the parolfactory nuclei and the tuberculum. This condition, which is so suggestive of, but not wholly similar to, the mammalian condition, has come about by a spreading and migration caudad of the lateral olfactory area, chiefly brought about by the great expansion of the dorsal pallium. The expansion of the pallium was accompanied by a great thickening of the lateral bundle and of the cell mass in which it was imbedded. The stretching of the lateral olfactory area accompanying the expansion of the pallium was followed by a collecting together of the olfactory centers into a ring around the striatal area. The striatum then, thickening and bulging outward, came to have its surface freed from the olfactory layer. That a complete ring of olfactory centers was formed around the striatal area was due to the connection which the medial olfactory tract retained with the nucleus of the lateral olfactory tract (fig. 56). This connection of the lateral olfactory nucleus with the medial tract and diagonal band has produced the characteristic bending of a part of the lateral olfactory tract to reach its nucleus in the amygdaloid region (fig. 60).

We have now to note how this condition in the turtle differs from that in the mammal. Although the gross appearance is much the same there is one essential difference, namely, that the striatal area has received a covering of general pallium, converting it into the insula. This has come about by the further expansion of the pallium which has pushed the pyriform lobe down over the striatal area so that in mammals it runs along the ventral border of the insula. Now a part of the lateral olfactory tract sweeps round in a curve to reach its nucleus in the tip of the temporal pole just as in the turtle. There is this difference, however, that the concavity of that sweeping curve is filled not by the striatal area but only by the tuberculum and the anterior perforate space.

The striatal area has thus been the scene of two important migrations of brain substance in the history of vertebrates. Between the fishes and reptiles the olfactory area has spread over the striatum and left its surface free again in the turtle. Between the turtle and mammals the general pallium has spread down over the striatum and left it covered by the insular cortex.

The hippocampal formation

With the expansion of the lateral brain vesicle and the formation of a caudal pole the massive hippocampal primordium in the roof of the selachian forebrain becomes spread out into a thin medial wall of the expanding vesicle. At the same time a part of the choroid roof of the median ventricle is carried out in the medial wall of the lateral ventricle and forms the choroid plexus extending from the interventricular foramen to the temporal pole. The relations of the hippocampus to the secondary olfactory centers in the medial wall have been fully discussed in previous papers and the history of the hippocampus above the reptiles is well known from the work of Elliot Smith and others.

The general pallium

The force which has brought about the changes in the olfactory centers mentioned above is the expansion of the general pallium. This is derived from the anterior end of the somatic sensory column (Johnston '10 c and '11 a) which early in vertebrate evolution extended up into the roof of the forebrain between the lateral olfactory area and the hippocampal primordium. This mass of cells received ascending fibers from the sensory centers in the thalamus and rapidly developed into an important collection of sensory and correlating centers for somatic relations. These centers proved of incomparable value to those vertebrates in which they were the more highly developed, through enabling them to extend the range of environment to which they could adapt themselves.

This expanding general pallium was located between the olfactory area and the hippocampus and was connected with its fellow of the opposite hemisphere by a commissure (corpus callosum) running through the substance of the hippocampus and in close relation to the hippocampal commissure. Under these conditions the expansion of the general pallium brought about great changes in the adjacent structures, as already noted. The first effect was an expansion distinctly in the dorsal or pallial portion of such a lateral forebrain vesicle as exists in the simpler selachian brains. This tended to produce a caudal pole pushing back over the stem or 'prethalamic' portion. To understand the formation of the caudal pole it is essential to see that the expansion began in this dorsal region and proceeded in the direction eventually assumed by the choroid fissure and the hippocampus. From such a beginning the stretching of the hippocampus and pyriform lobe and the migration of the olfactory area over the striatum all are readily understood. The further expansion of the pallium and the differentiation of special centers in it must be the subject of future work for which the present is preparatory.

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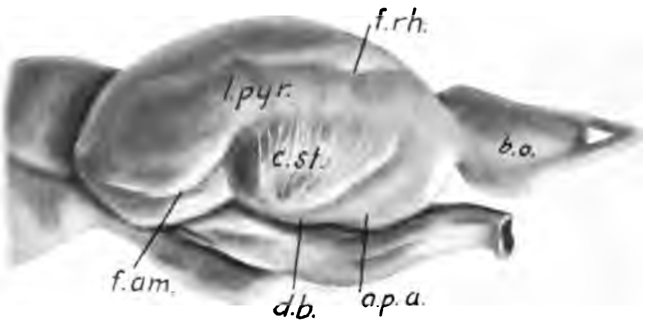
REFERENCE LETTERS

a.p., area parolfactoria
a.p.a., area perforata anterior
b.o., bulbus olfactorius
c.a., commissura anterior
c.h., commissura hippocampi
c.i., capsula interna
ch.op., chiasma opticum
c.post., commissura posterior
c.p.a., commissura pallii anterior
crus, crus cerebri or lateral forebrain bundle
c.st., corpus striatum
d.b., diagonal band of Broca
dec.po., decussatio postoptica
d.v.r., dorsal ventricular ridge
f.am., fissura amygdaloidea
fasc.m., fasciculus marginalis
f.chor., fissura chorioidea
fi., fimbria
f.pc., fasciculus praecommissuralis
f.o., formatio olfactoria
f.p., fissura prima
for.i., foramen interventriculare
f.rh., fissura rhinalis
gl., olfactory glomerulus
g.p., general pallium
gr., granule cells and layer
g.s., gyrus subcallosus
h., hippocampus
hy., hypothalamus
i.C., islands of Calleja
l.pyr., lobus pyriformis
l.t., lamina terminalis
m.fb.bdl., medial forebrain bundle
mi., mitral cells and layer
n.c., nucleus caudatus
n.d.b., nucleus of the diagonal band
n.l., nucleus lentiformis
n.med.a., large-celled medial nucleus of the amygdaloid complex
n.o., nervus olfactorius
n.o.a., nucleus olfactorius anterior
n.p.l., nucleus parolfactorius lateralis
n.p.m., nucleus parolfactorius medialis
n.rot., nucleus rotundus
n.sup., nucleus supraopticus
n.tr.olf.lat., nucleus of the lateral olfactory tract
olf.proj., olfactory projection tract
pa., pallium
pa.th., pallial thickening
p.c., layer of peripheral cells in the pallium
p.h., primordium hippocampi
p.o. pedunculus olfactorius
r.p., recessus praeopticus
r.n., recessus neuroporicus
r.s., recessus superior
s.en., sulcus endorhinalis
s.f-d., sulcus fimbrio-dentatus
s.l., sulcus limitans hippocampi
s.m., stria medullaris
s.p.a., substantia perforata anterior
s.t., stria terminalis
sub., subiculum cornu ammonis
s.v.d., dorsal ventricular sulcus
s.v.m., middle ventricular sulcus
s.v.v., ventral ventricular sulcus
thal., thalamus
t.o., tuberculum olfactorium
t.p., temporal pole
t., tectum mesencephali
tr.d.b., tract of the diagonal band
tr.olf.lat., lateral olfactory tract
tr.olf., tractus olfactorius
tr.olf.med., medial olfactory tract
tr.op., tractus opticus
v.III., third ventricle
v.l., ventriculus lateralis

Fig. 1 Lateral surface of forebrain. Chief features described in text. The fibers in the striatal area are readily seen both in the fresh brain and in brains prepared in bichromate. These fibers are equivalent to the mammalian internal capsule, but the cortical covering of the insula is wanting. The pyriform lobe shows some cross ridges which probably correspond to bundles of internal capsule fibers and thickenings of the lateral border of the pallium. The amygdaloid fissure was especially well developed in this brain. The whole brain never shows the rostral end of the sulcus endorhinalis as well as sections do.



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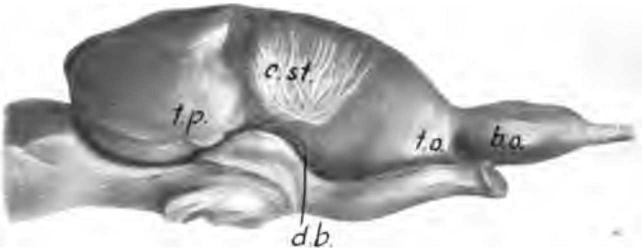

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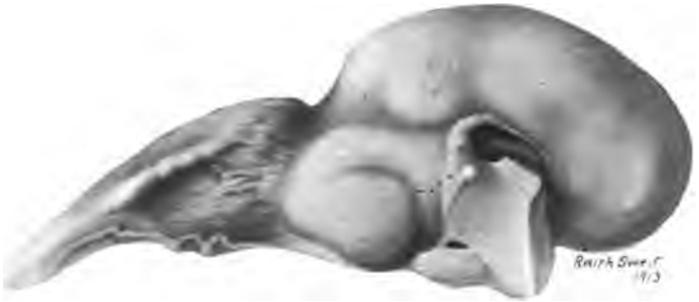

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Fig. 2 A ventral view of the same brain as in figure 1. The diagonal band and the fibers imbedded in it are readily visible in the entire brain, although the fiber-bundles are much more delicate than those of the internal capsule.

Fig. 3 A medial view of another specimen. (From Johnston '13 b, fig. 20.) The fissura prima curves forward over the parolfactory area. The fimbrio-dentate sulcus runs longitudinally above it. The body between is the primordium hippocampi. Between the fissura prima and the lamina terminalis the diagonal band rises apparently to join the hippocampal primordium directly. The broken line indicates the boundary of the primordium as determined by internal structure. The portion of the diagonal band seen on this surface of the brain is the homologue of the gyrus subcallosus. This figure shows the prominence of the rostral end of the pallium due to the pallial thickening.

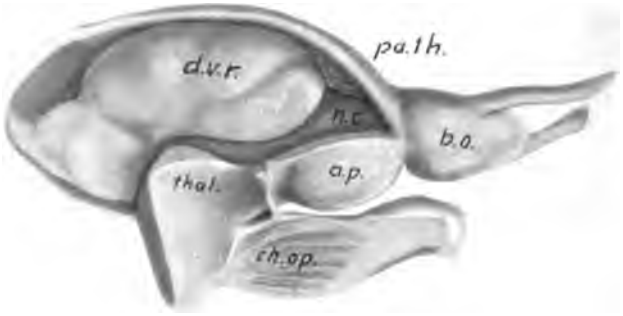
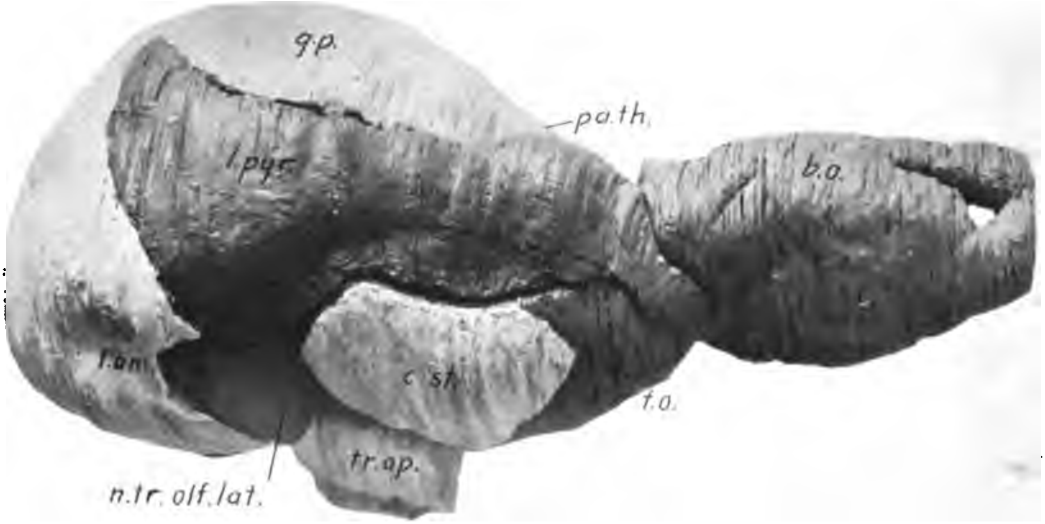


Fig. 4 A forebrain from which the entire medial wall of the pallium has been cut away, seen from the medial direction. The large dorsal ventricular ridge is divided into a chief lobe and a basal lobe by a shallow groove. The bifurcation of the middle ventricular groove is not well shown. One branch descends into the temporal horn of the ventricle; the other runs for a short distance in the bottom of the shallow groove on the dorsal ridge. Compare figure 10. This brain shows an unusual prolongation of the basal part of the dorsal ridge into the caudal pole. The part of the striatum seen here is the head and tail of the caudate nucleus.

Figs. 5 to 10 These are photographs of a model of the right half of the forebrain made by the method of plate reconstruction. The method of building up and dissecting the model is explained in the text. Before photographing, the outer surface of the model was lightly smoothed and then painted in two colors. All the olfactory centers including the hippocampus were painted a light yellow-green. The somatic structures, striatum and general pallium, were painted light blue. In the photographs all the olfactory centers have a dark tone. The ventricular surfaces and the cut surfaces have received no treatment whatever. The hypothalmic region was not completed.

Fig. 5 Lateral surface of the model. The dorsal border of the pyriform lobe is sharply marked through its whole length. The caudal border is only arbitrarily fixed. A broken line marks the limit between the large-celled and small-celled parts of the pyriform lobe. The olfactory part of the amygdaloid eminence appears depressed owing to its color and to its being in shadow. Compare figure 8. The structures surrounding the striatal area constitute a continuous ring of small-celled olfactory nuclei.

Fig. 6 Medial view of the model. The lip of the choroid fissure and the lamina terminalis are painted white. The model is cut between the hippocampal commissure and the anterior commissure, so that the commissure may be removed with the whole hippocampal formation. A broken line indicates roughly the boundary between the hippocampus proper and the subiculum. Probably too large an area is included in the subiculum at the anterior end. The somatic pallium should have been carried farther forward here in constructing the model. Note the oblique position of the peduncular constriction. Note also the fissura prima, the fimbrio-dentate sulcus and the moulding of the caudal pole around the thalamus and midbrain.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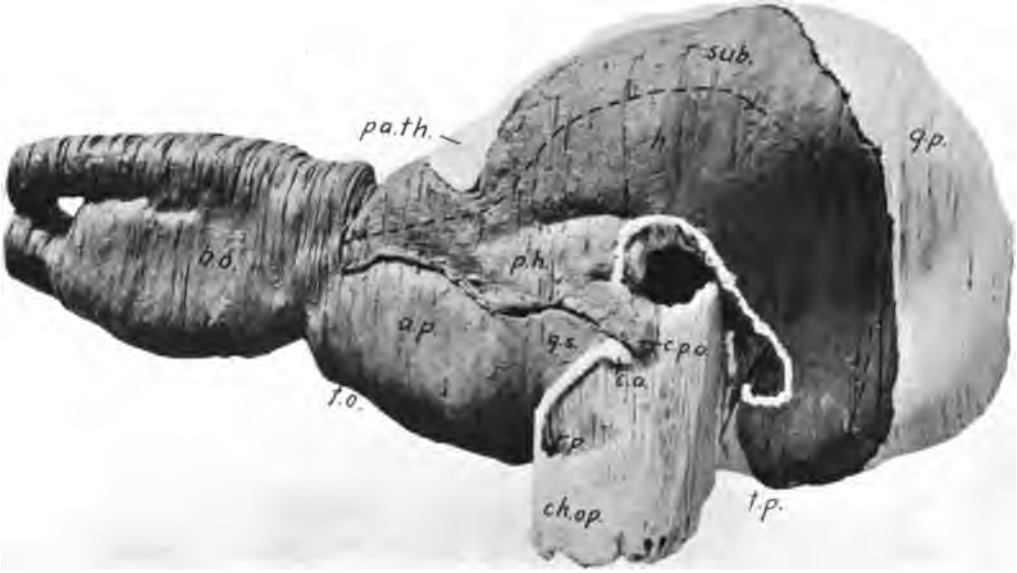
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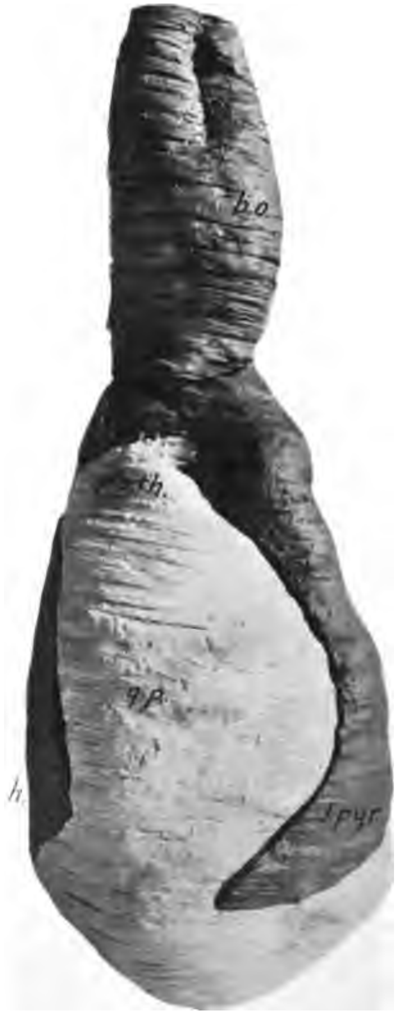


Fig. 7 Dorsal view of the model. This requires no comment except to point out that the general pallium should have been continued somewhat farther forward at the expense of the subiculum cornu ammonis.

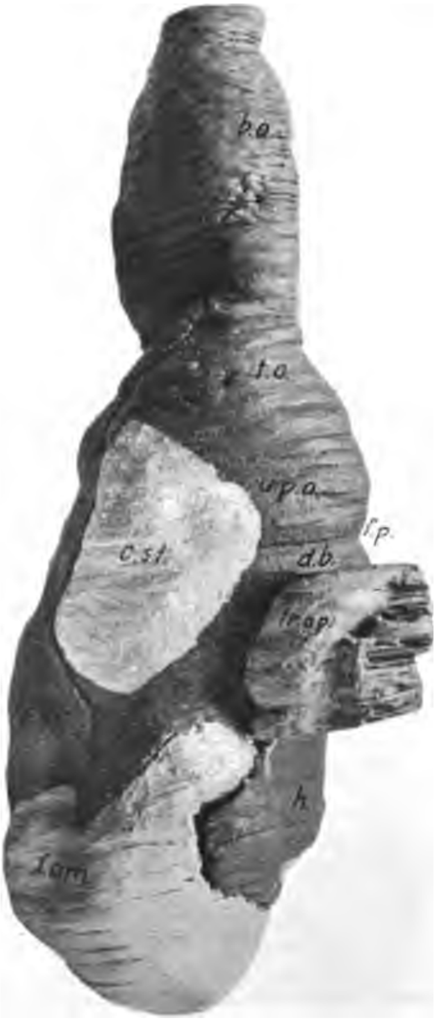


Fig. 8 Ventral view of the model. This view shows well how the striatal area is completely surrounded by olfactory centers. It also shows that the general pallium extends into the amygdaloid prominence of the temporal pole so as to intervene between the hippocampus and the secondary olfactory nuclei. The temporal pole of the hippocampal formation has no direct relation with the secondary olfactory nuclei on the basal aspect of the brain.

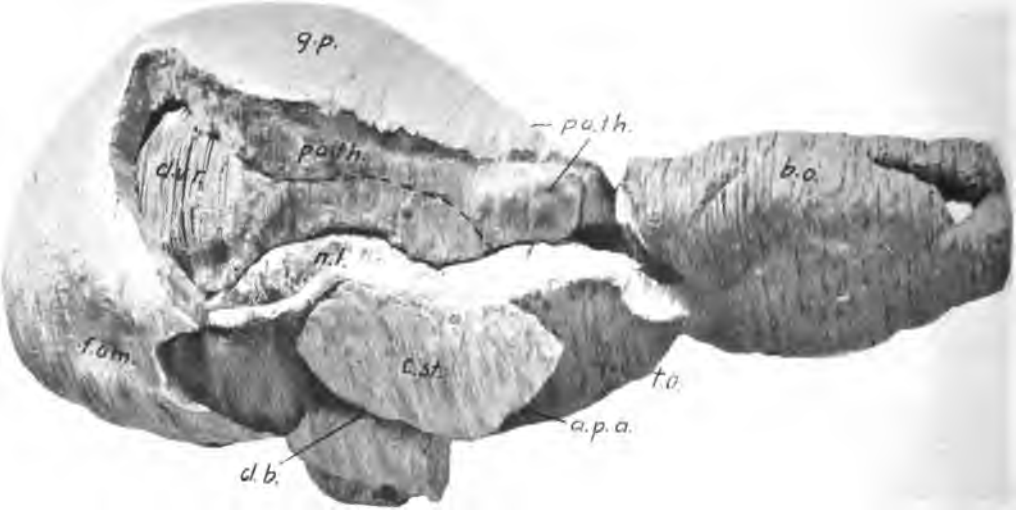


Fig. 9 Lateral view of the model with the pyriform lobe removed. At both its rostral and caudal ends the pyriform lobe forms the whole thickness of the wall over small areas, so that the ventricle is opened in this photograph. Through the greater part of its length the pyriform lobe overlies the thickened lateral border of the pallium and the dorsal ventricular ridge. A broken line marks the boundary between the pallium and this ridge. Along this line a cell-free zone separates the pallium from the ridge but the two can not be dissected apart owing to the continuity of their cell masses in the region of the amygdaloid fissure. The thickening of the lateral border of the pallium which causes the bulging out of the pyriform lobe near its rostral end is clearly seen in this photograph.

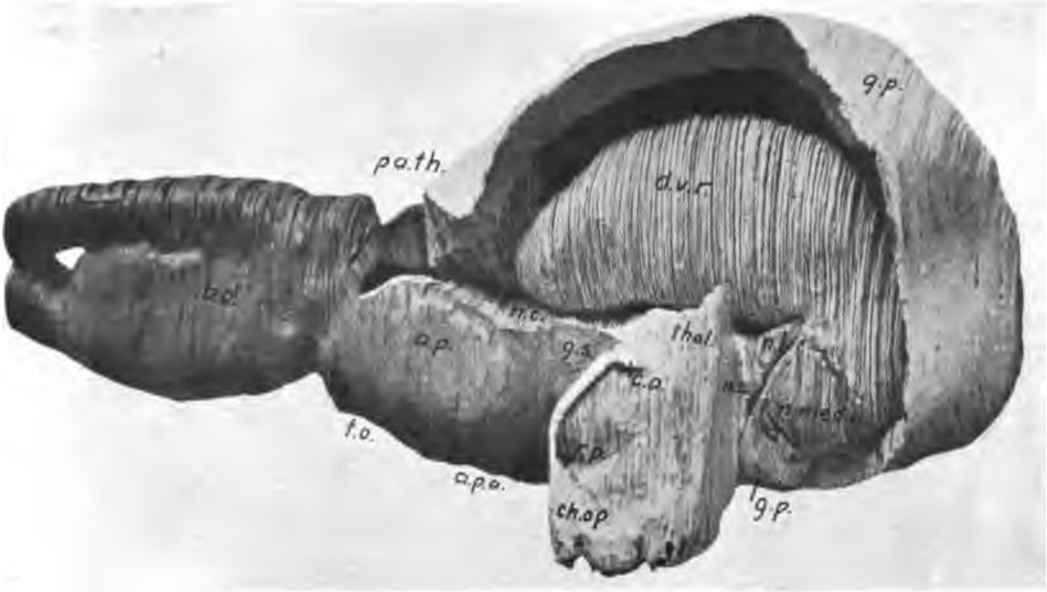


Fig. 10 Medial view of the model with the hippocampus removed. The pallial thickening is perhaps better seen here than in the dissected brain (fig. 4). That part of the thalamus which appears behind the anterior commissure is continuous laterally with the tail of the caudate. The ventricular ridge and general pallium are separated from the striatum by a cut which follows the middle ventricular groove to the end of its dorsal branch, and by a cut which runs diagonally downward and forward from the end of the first one and disappears behind the hypothalamus in this view. The bifurcation of the middle ventricular groove is seen just behind the upper angle of the thalamus. From this point the temporal branch descends into the temporal horn of the ventricle. Its course forms a letter X with the second cut above described. Following the course of this groove is the small ridge described in the text as imbedding part of the stria terminalis related to the basal lobe of the ventricular ridge. From this ridge the bundle goes forward in the caudate. The tail of the caudate barely appears in this figure behind the thalamus (n. c.) and bounded by the temporal branch of the middle groove. The thickening and fusion of the caudate with the nucleus of the lateral olfactory tract in the amygdaloid complex lies just lateral to this point. It is in this region that a great hypertrophy of the common caudate-amygdaloid mass produces the 'epistriatum' in the lizards. The triangular area between the two cuts above described belongs to the lentiform nucleus. Note that the general pallium is continuous with the lentiform nucleus through the basal lobe of the ventricular ridge (medial nucleus of amygdaloid complex).

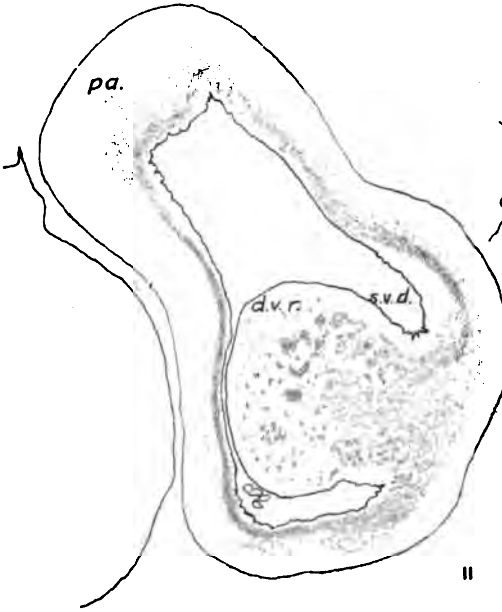
Figs. 11 to 30 Transverse sections of the right hemisphere drawn from sections stained to demonstrate the size and grouping of the cell bodies. The contrasts and lines of separation are not exaggerated. Magnification, 17.5 diameters. The position of each of these sections is indicated in figure 58.

Fig. 11 Section through the base of the dorsal ridge. The dense layer in the medial wall of the pallium is in part hippocampus. The rest of the section belongs to general pallium, with which the ridge is in connection.

Fig. 12 Section a short distance behind the choroid fissure. The dense pallium in the medial wall is hippocampal. The dorsal ridge appears as an infolding of the general pallium. The pyriform lobe presents a confused arrangement of its cells.

Fig. 13 Section at the caudal border of the choroid fissure and the base of the amygdaloid fissure. Hippocampal formation appears both above and below the choroid fissure. The large-celled nucleus appears on the ventricular surface of the basal lobe of the great ventricular ridge. The nucleus of the lateral olfactory tract appears near the base of the amygdaloid fissure. Between them the general pallium is continuous with the central core of the ventricular ridge. Note the small ridge below, which contains the stria terminalis bundle. From this level forward the subiculum cornu ammonis is a conspicuous feature.

Fig. 14 Section through the extreme rostral end of the temporal horn of the ventricle. Hippocampal formation has disappeared below the choroid fissure. The large-celled nucleus surrounds this part of the ventricle. The nucleus of the lateral olfactory tract has grown larger and the caudate nucleus appears and is fused with it. The nucleus lentiformis begins to take shape.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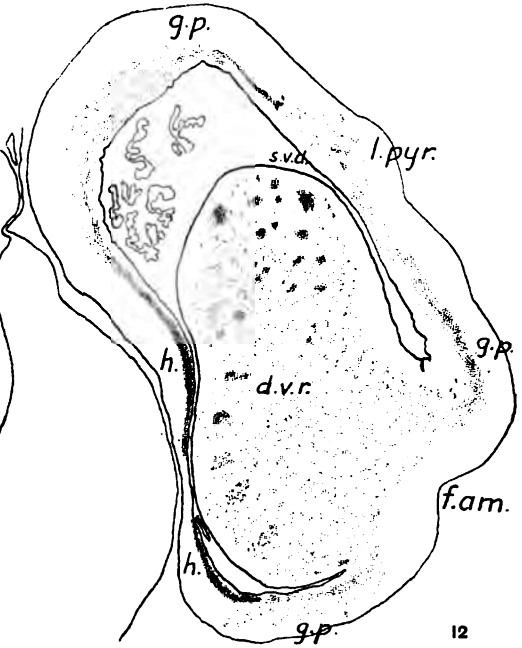
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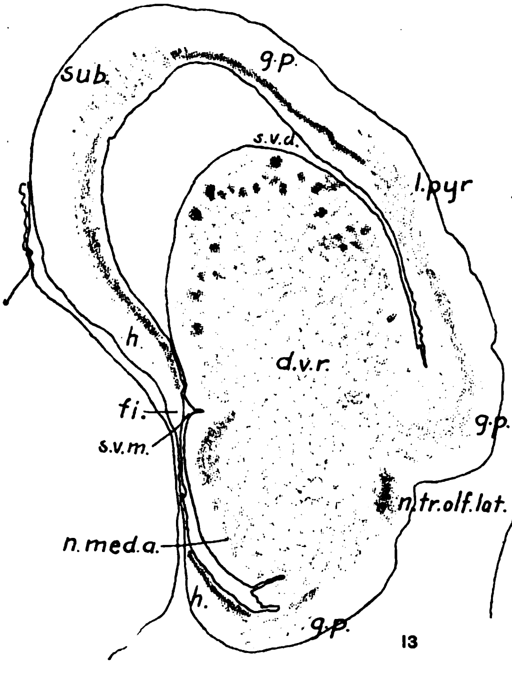
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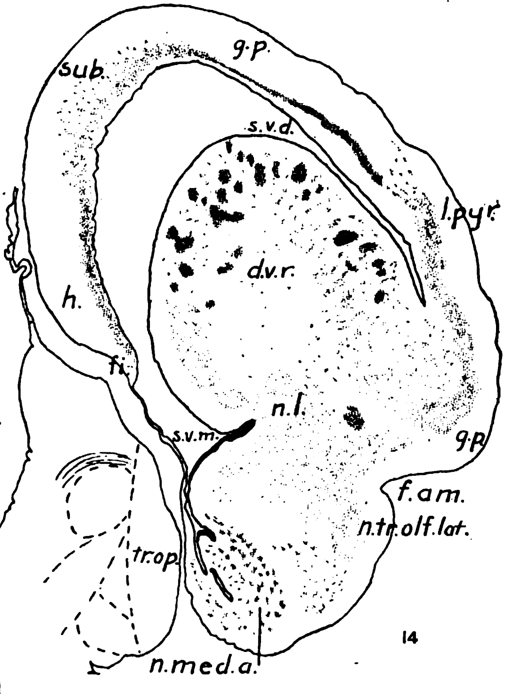
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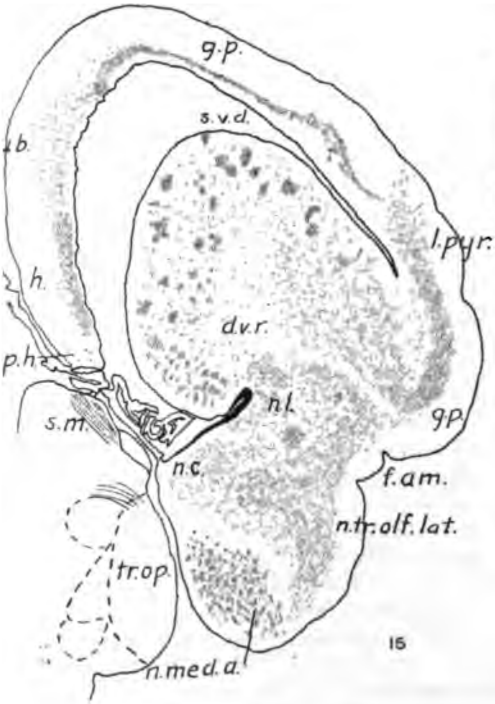
Fig. 15 Section at the base of the amygdaloid fissure. The general pallium still makes connection with the ventricular ridge and intervenes between the pyriform lobe and the nucleus of the lateral olfactory tract. This last is now larger and merges broadly with the caudate. The lentiform nucleus shows some dense collections of cells.

Fig. 16 Section through the stria medullaris. This is about the largest and densest part of the small-celled area of the amygdaloid complex, consisting of the caudate and nucleus of the lateral olfactory tract. The great enlargement of this body produces the 'epistriatum' in lizards. The olfactory projection tract and part of the stria terminalis are related to this (compare fig. 49) while the stria medullaris enters the neuropile surrounding the rostral end of the medial nucleus. Note the rapid thickening of the lateral border of the dorsal pallium in figures 15 and 16. An angle of the general pallium connected with the ventricular ridge still intervenes between the pyriform lobe and the nucleus of the lateral olfactory tract.

Fig. 17 Section through the extreme tip of the temporal pole or amygdaloid prominence and through the caudal part of the junction of thalamus and striatum. The nucleus lentiformis is larger and the nucleus of the lateral olfactory tract appears divided. The upper portion will in the next figure join the pyriform lobe. The core-nucleus of the ventricular ridge is now well defined. Note the olfactory projection tract curving over the crus.

Fig. 18 Section through the commissures just rostral to the inter-ventricular foramen. The crus bends laterad into the lentiform nucleus. It separates the caudate from the nucleus of the diagonal band which is a continuation of the nucleus of the lateral olfactory tract. Note the continuity of the caudate with the nucleus anterior region of the thalamus in figures 17 and 18. Note also the rapid enlargement of the primordium hippocampi in these figures as it approaches the level of the commissure. For the disposition of the commissures compare figures 32, 48 and 52.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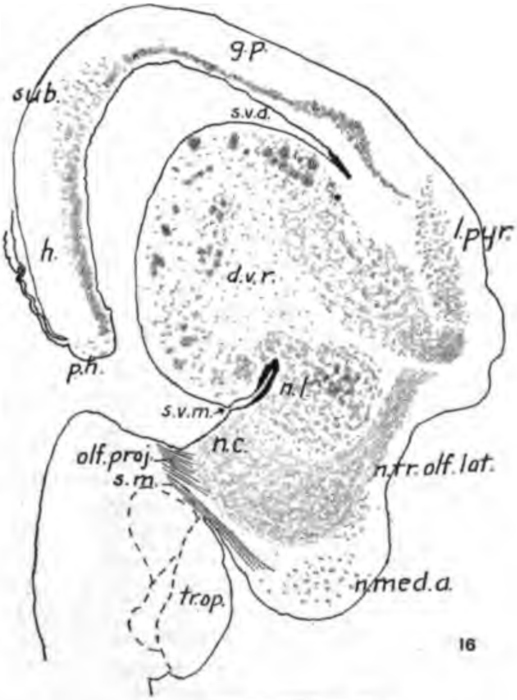
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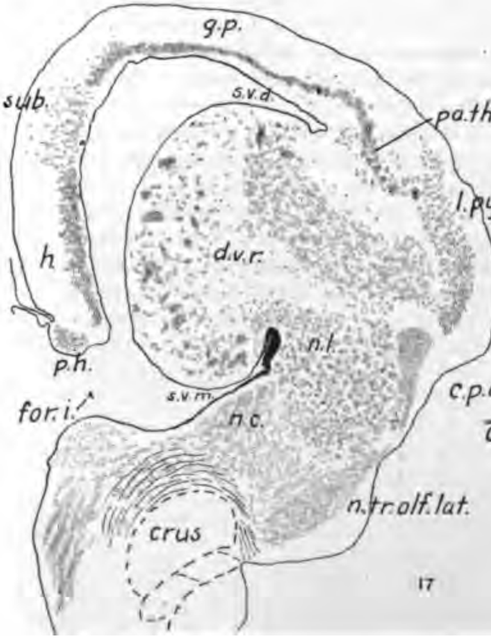
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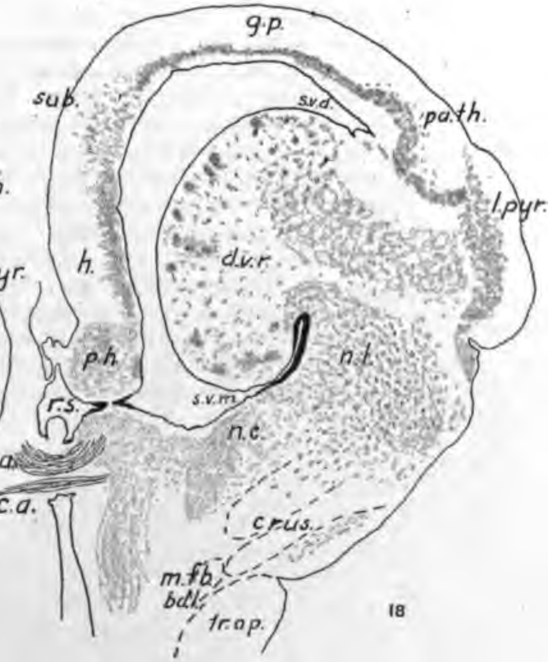
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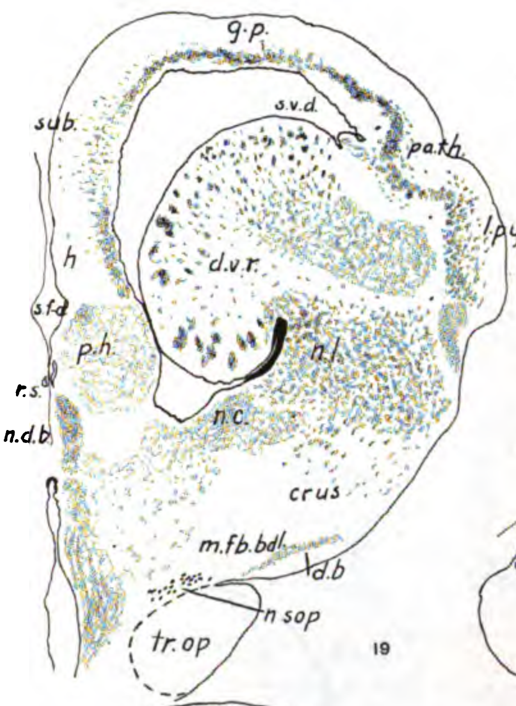
Fig. 19 Section in front of the commissures and behind the optic chiasma. The lentiform nucleus is large; the crus occupies the clear space below it and sends fascicles up through it (internal capsule). The nucleus of the diagonal band forms a thin cell layer between the fiber layer of that band and the medial forebrain bundle. The caudate is small. The upper end of the nucleus of the diagonal band appears here in the gyrus subcallosus adjacent to the hippocampal primordium and the recessus superior. Note the distinct large- and small-celled portions of the pyriform lobe. Also the general increase in thickness and more open formation of the cell layer of the dorsal pallium.

Fig. 20 Section through the optic chiasma. The ventricular ridge grows smaller and the definite pallial thickening begins. The hippocampus proper grows narrow. The hippocampal primordium is only apparently fused with the parolfactory nuclei, as explained in the previous paper. The diagonal band and its nucleus move to the angle between the basal and medial walls. The sulcus endorhinalis separates the small-celled from the large-celled part of the pyriform lobe. The lentiform nucleus is quite superficial in this and several following figures. The clear zones above and below the core-nucleus of the ventricular ridge and between that and the pyriform lobe are the spaces through which internal capsule fibers run to the ridge and the general pallium.

Fig. 21 Section at the level of the gyrus subcallosus. The changes noted in the last section have been carried farther. The lentiform nucleus shows rows of cells due to the arrangement of bundles of internal capsule. In this and figure 22 the diagonal band nucleus merges with the superficial layer of the anterior perforate space and tuberculum olfactorium.

Fig. 22 Section through the anterior end of the dorsal ventricular ridge. The core-nucleus is here divided into two areas by pressure from the pallial thickening. This thickening is the most conspicuous feature of the section. Note the development of a layer of peripheral cells in figures 20, 21 and 22. Compare figure 40. The caudate begins to enlarge and the lentiform to decrease.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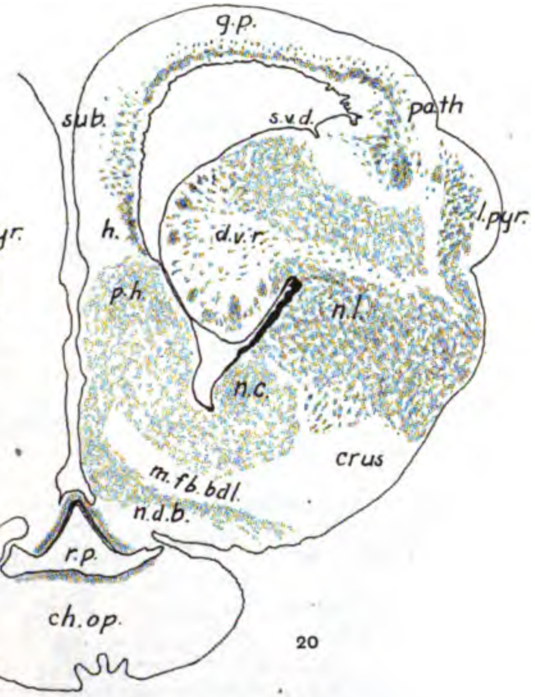
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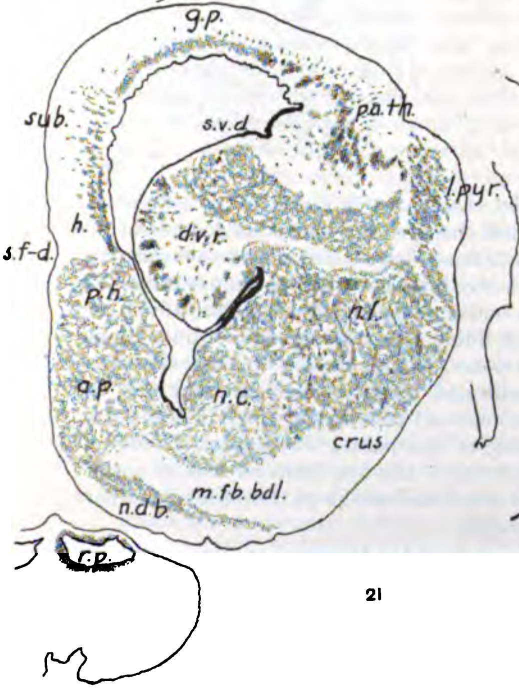
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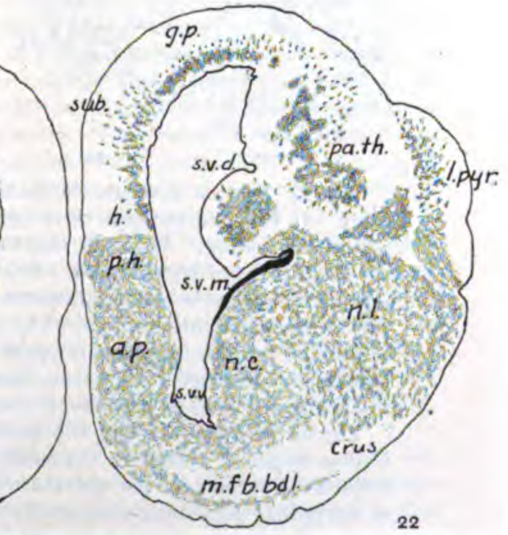
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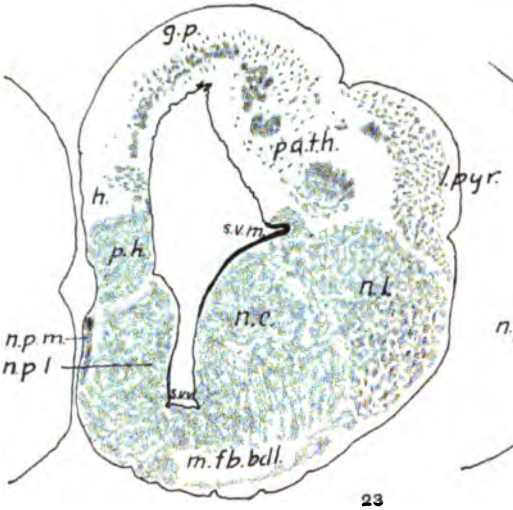

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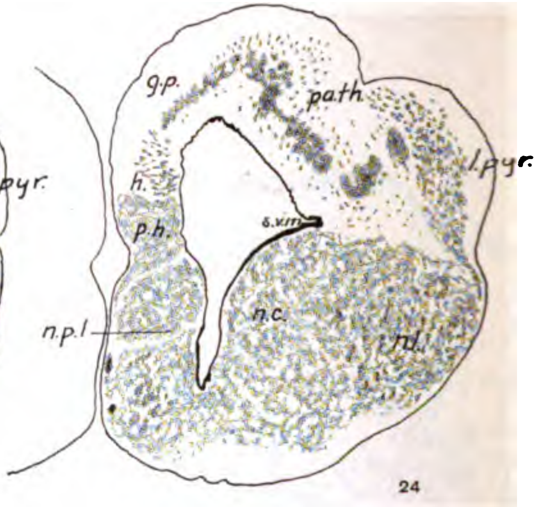

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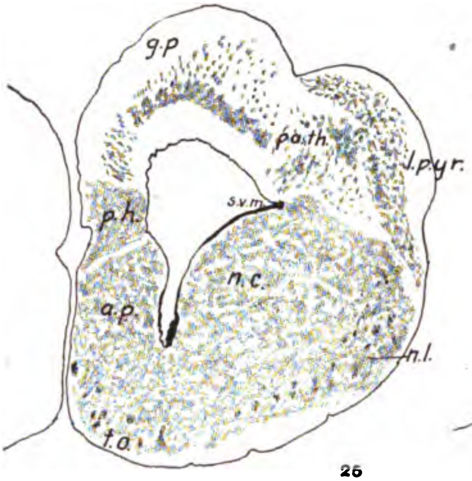

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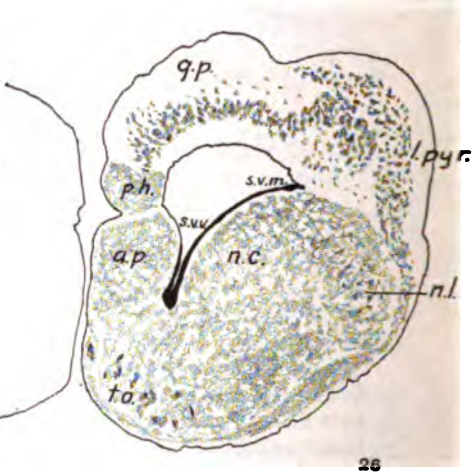

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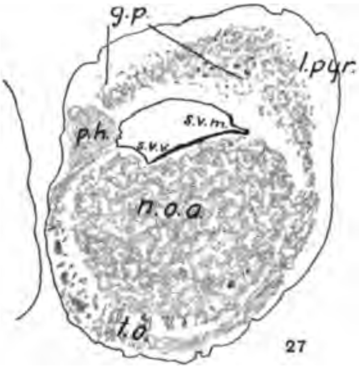
Fig. 23 Section through the pallial thickening in front of the ventricular ridge. In this and the next three figures the pyriform lobe is bulged out by the pallial thickening. Although the lentiform nucleus still meets the ventricle, the caudate is greatly enlarged. From here forward the hippocampus is scarcely if at all recognizable. The subiculum continues between the general pallium and the hippocampal primordium. The dividing line between primordium hippocampi and parolfactory area is again clear. In this section the ventral ventricular groove reaches its greatest depth and width. Forward from this the thickening of caudate, parolfactory nucleus and tuberculum nearly obliterate it.

Fig. 24 Section through the middle of the pallial thickening. Islands of Calleja begin to appear in the medial wall. The lentiform nucleus is pushed toward the surface by the caudate. For a detail of the pyriform lobe and part of the pallial thickening compare figure 40.

Fig. 25 Section through the caudal part of the tuberculum olfactorium and the head of the caudate. Note the high dorsal prominence caused by the pallial thickening. This scarcely shows satisfactorily in the figures of the whole brain or model. A part of the apparent thickness of the cell layer is due to the section being slightly oblique to the curvature of the dorsal pallium but the appearance of a distinct peripheral layer of cells is not due to this. Islands of Calleja are grouped in the medio-basal angle. Dark masses farther laterad are merely clumps of large cells in the lentiform nucleus.



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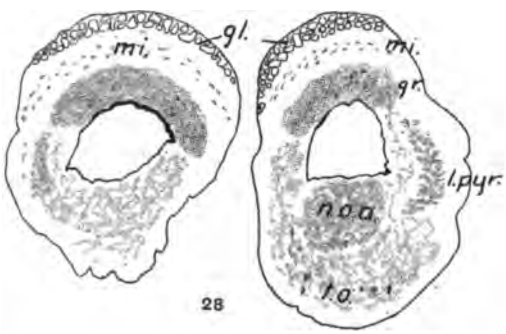

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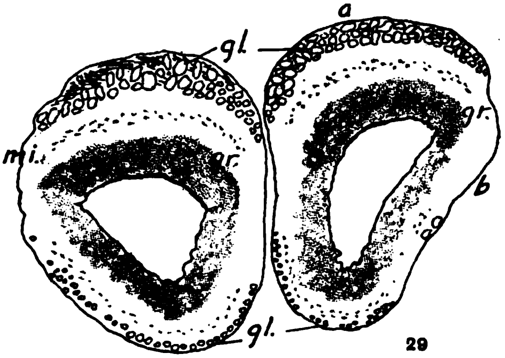

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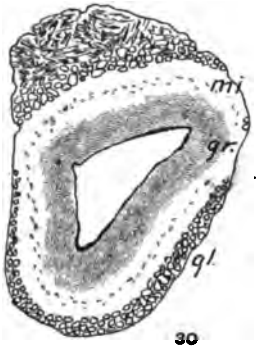

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Fig. 26 Section a short distance in front of the last. It shows the rostral end of the lentiform nucleus and the spreading of the small-celled part of the pyriform lobe over the striatum as the superficial layer of the tuberculum. The whole width of pallium in figs. 25, 26 has the characters of the thickening.

Fig. 27 Section through the rostral part of the tuberculum. The section passes just behind the peduncular constriction dorsally. Olfactory formation begins to appear 225 microns farther forward. The head of the caudate together with the deep or lateral parolfactory nucleus form one rounded mass covered externally by tuberculum. This is the nucleus olfactorius anterior of authors. Islands of Calleja are in the deep layer of the tuberculum. The tuberculum is continuous with the medial parolfactory nucleus and with the pyriform lobe. The pyriform lobe spreads medially in dorsal wall. General pallium is still present toward medial border and in some clusters of cells beneath pyriform lobe.

Fig. 28 Section through the olfactory peduncle. Both sides are shown, the left side being sectioned near the rostral end of the oblique peduncle, the right side near the caudal end of the peduncle. On the right side fully developed olfactory formation appears in the roof; in the lateral wall the pyriform lobe; below, the end of the nucleus olfactorius anterior and the tuberculum.

Fig. 29 Section through the caudal part of the olfactory bulb. Note the elliptical form of section on the right, the greater number of cells where olfactory glomeruli are present and the distribution of mitral cells with reference to the glomeruli. *a*, *b*, regions of this section which are drawn in figure 31.

Fig. 30 Section through the anterior part of the right bulb. Note the triangular form of section and the fact that the dorsal root of the olfactory nerve is just entering the bulb. The bulb is nearly surrounded by glomeruli and the mitral cell layer is continuous except for a short space on the lateral surface where glomeruli are absent.

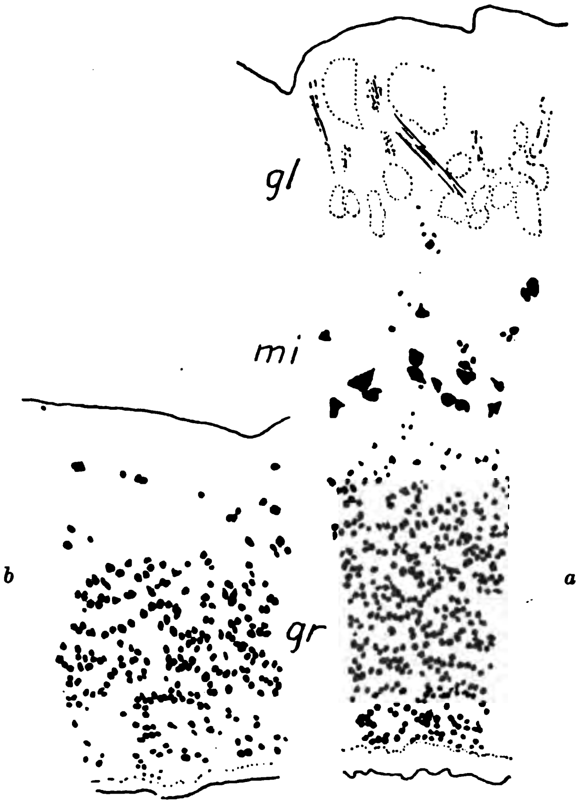
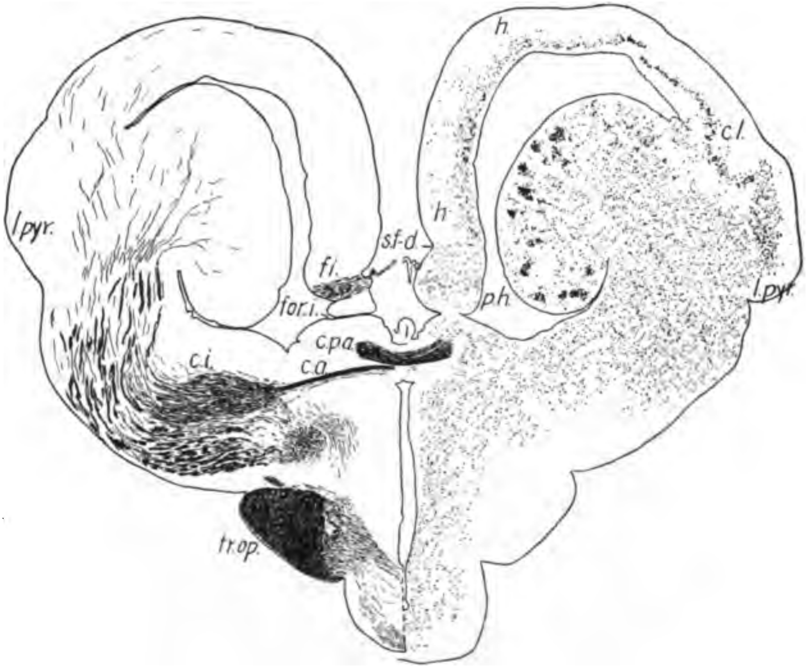


Fig. 31 Two portions from the section drawn in figure 29 to show the modification of the structure of the olfactory formation due to the presence of glomeruli. The part of the section drawn in *a* and *b* respectively is indicated by the same letter in figure 29. Where glomeruli are present the granule cell layer is considerably thicker and more dense and the layer of large mitral cells is added. Magnification 110 diameters.

Fig. 32 Transverse section at the level of the commissures. From Johnston '13b, fig. 17. The right side of the drawing was taken from a section adjacent to that from which figure 18 of the present paper was drawn. The left side was drawn from a Weigert series. The crus shows the two chief bundles described in the text. To what extent the fascicles of the two bundles intermingle as they are distributed to the pallium and the ventricular lobe it is impossible to say from sections of normal brains. The relation of the anterior commissure to the internal capsule is better shown in figure 48. Medial to the crus appear two other bundles. The darker one is the medial forebrain bundle, the lighter is the olfactory projection tract.

Fig. 33 A section similar to the last, taken at the level of the stria medullaris. From Johnston '13b, fig. 18. The two bundles of the crus are separated by a notch medially. The medial forebrain bundle is ventral to the olfactory projection tract and in contact with the postoptic decussation. The lower part of the fiber area beneath the letters *c.i.*, belongs to the olfactory projection tract. The stria medullaris is more superficial.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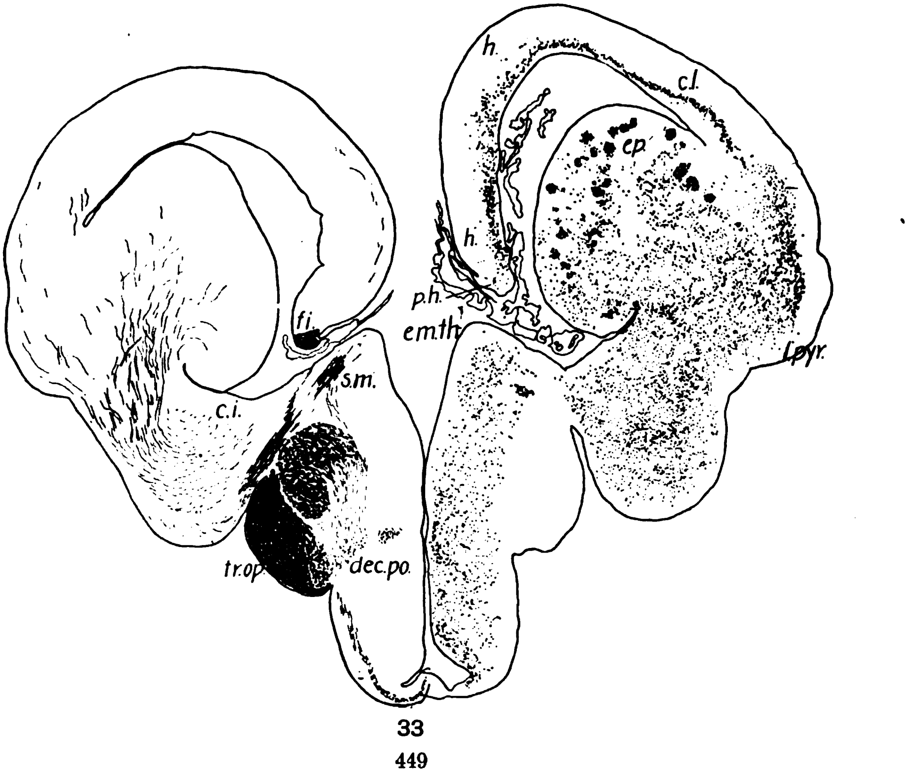
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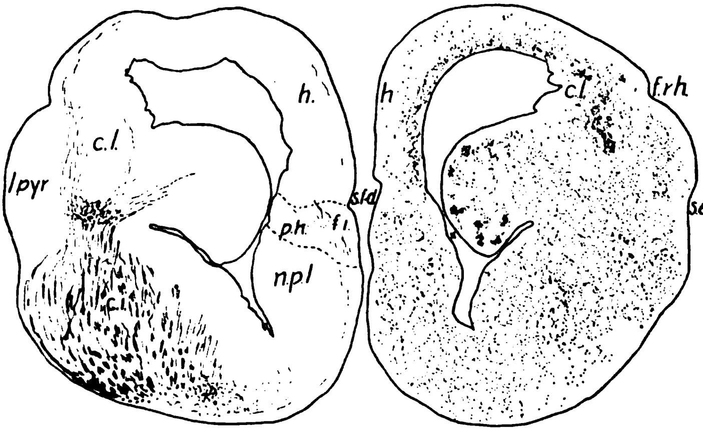

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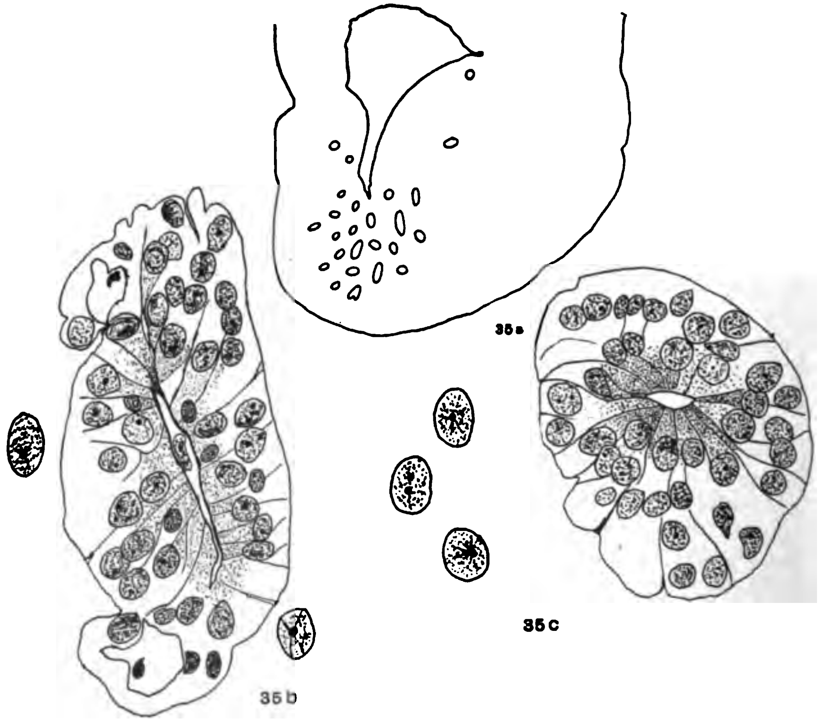

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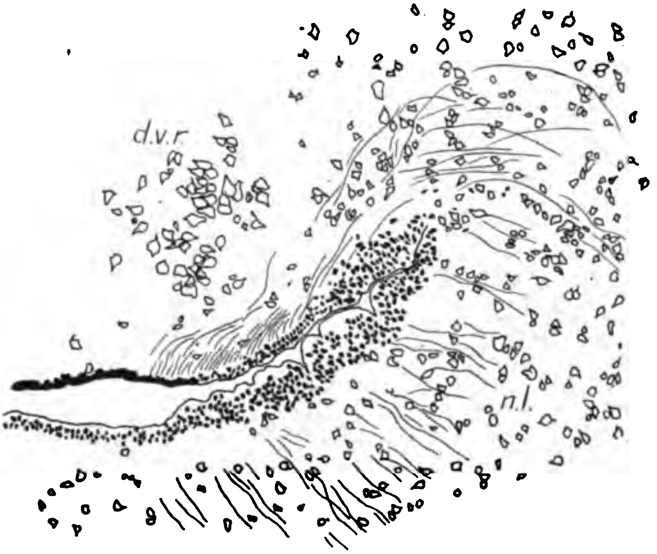

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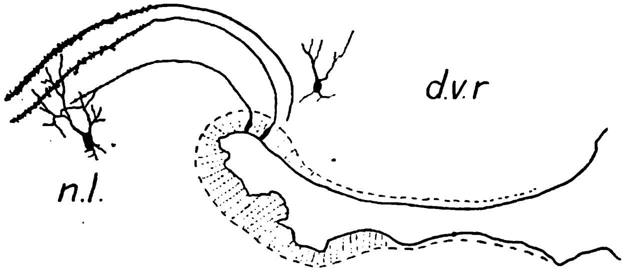
Fig. 34 A section similar to the last, taken near the rostral end of the ventricular ridge. The internal capsule fibers are related largely to the pallial thickening (*c.l.*). The medial part of the fiber bundles below belongs to the medial forebrain bundle. Its fibers are related only to the caudate and olfactory nuclei.

Fig. 35 Vesicular structures in the caudate nucleus. *a*, outline of a transverse section to indicate the position in which such vesicles are found. This is nearly at the same level as figure 25.. Vesicles are found both rostral and caudal to this level. *b*, an oblique section of a vesicle the lumen of which contains a flattened nucleus of unknown character. Two blood corpuscles are seen. Vesicles often have capillaries closely connected with them. *c*, a transverse section of a vesicle with very sharply defined lumen. Note the granular contents of the inner ends of the cells. There are also faintly stained granules in the lumen. The isolated nuclei in *b* and *c* are nuclei of nerve cells lying near the vesicles. Magnification in *b* and *c*, 625 diameters.

Fig. 36 Section of the thickened ependyma surrounding the middle ventricular groove including a part of the lentiform nucleus. The nerve cells are drawn in outlines, the nuclei of the ependyma cells in black. The lines represent the peripheral processes of the ependyma cells. From the same section as figure 16. Magnification 112 diameters.



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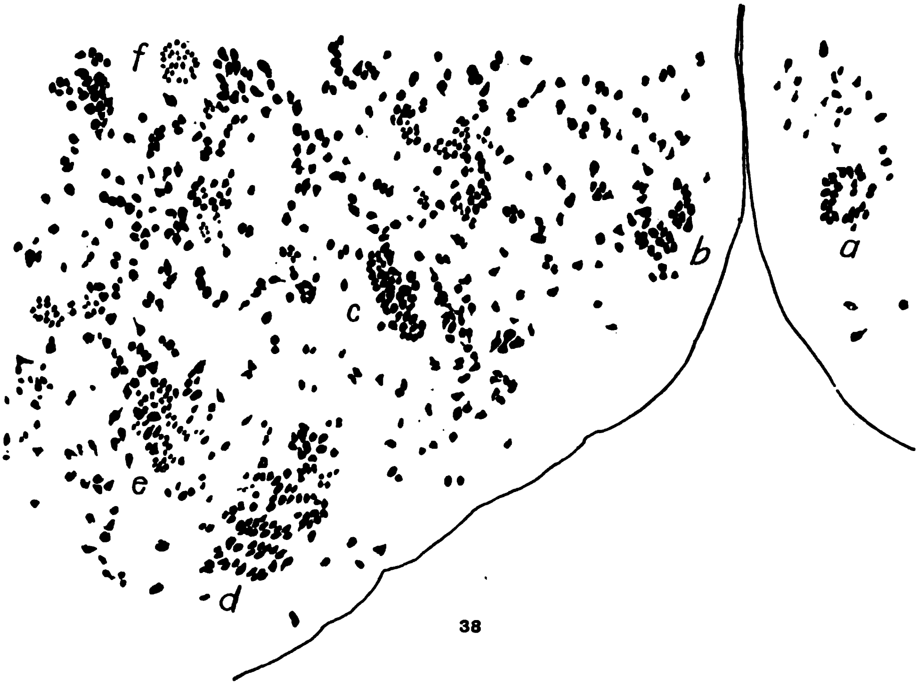

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Fig. 37 Same region as figure 36 but on the left side of the brain. A drawing from a Golgi section to show the ependyma cells in the dorsal lip of the middle ventricular groove, and their curved processes.

Fig. 38 Transverse section through the tuberculum olfactorium to show the grouping of cells in the islands of Calleja. From a section adjacent to that shown in figure 24. Magnification 110 diameters. The medio-basal angle of the left side and a small part of that of the right side are included. *a*, *b*, *c*, *d*, typical islands. *e*, an island in which larger and small cells are mingled. In some islands similar to this the small cells definitely show the vesicular arrangement with lumina. *f*, a vesicle similar to those shown in figure 35. Only the nuclei are drawn.

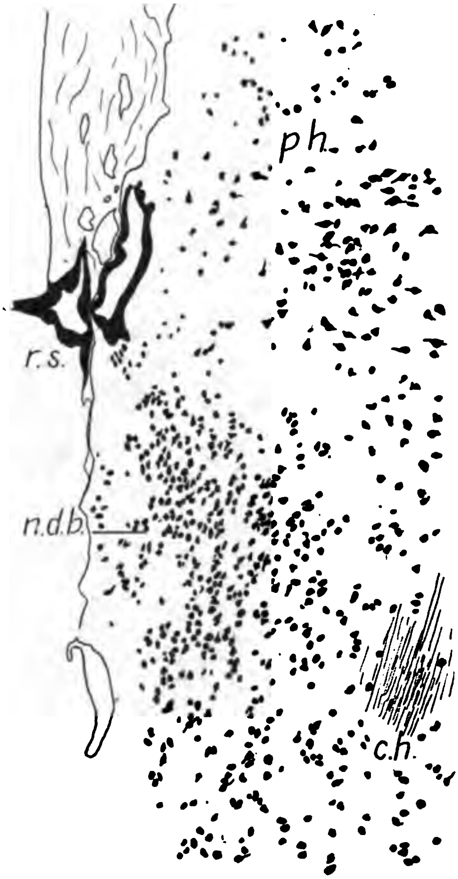


Fig. 39 Transverse section through the upper end of the gyrus subcallosus, showing the dense nucleus of the diagonal band close to the recessus superior. From the same section as figure 19. The position of the hippocampal commissure shows that this nucleus pushes up near the medial surface above the level of the commissure. This is shown in the model, but the ventral border of the hippocampal primordium in front of the commissure curves dorsally still more than is shown in the model (fig. 6). Note how much smaller these cells are than those of the adjacent hippocampal primordium. Indeed these are among the smallest cells in the forebrain. Magnification 110 diameters.

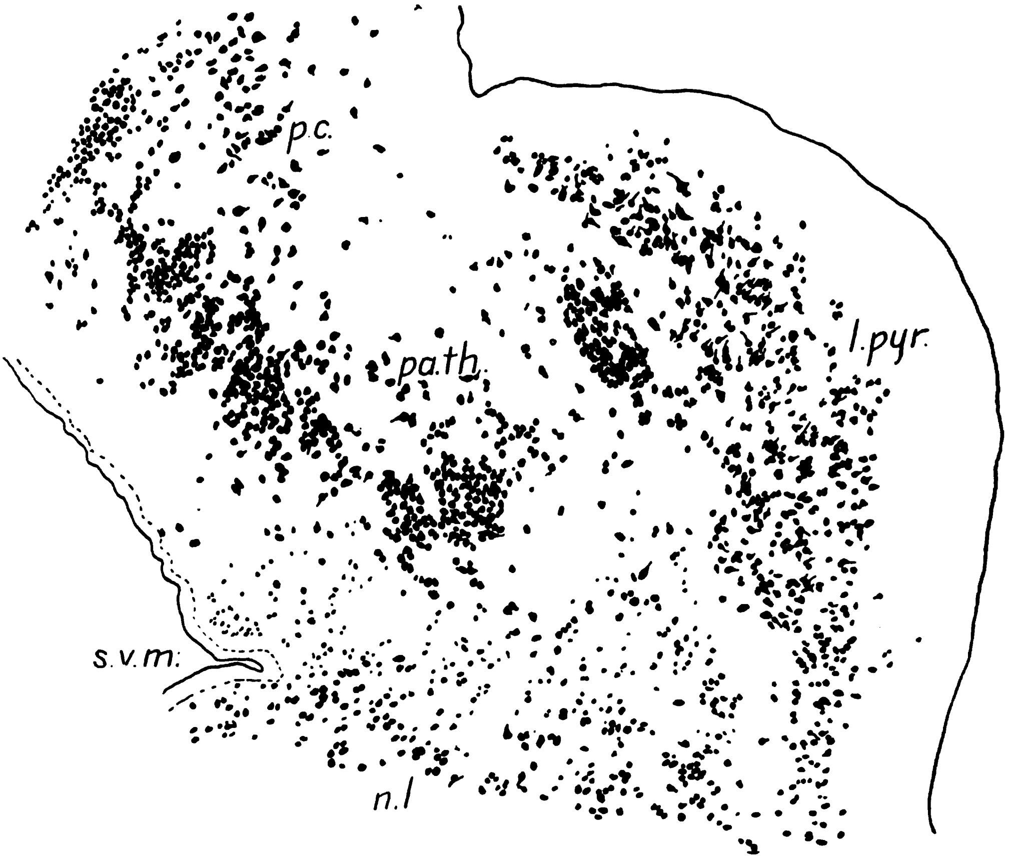


Fig. 40 Transverse section through the pyriform lobe and the pallial thickening to show the form and grouping of the cells. From the same section as figure 24. Magnification 70 diameters. In the upper part of the figure enough of the pallium is drawn to show the importance of the peripheral layer of cells. The deeper cells are smaller and so closely crowded that their processes seldom show at this magnification. The thick dendrites of the larger cells in the pyriform lobe, however, are clearly seen. It is evident that the large cell clusters belong to the pallium and not to the pyriform lobe.

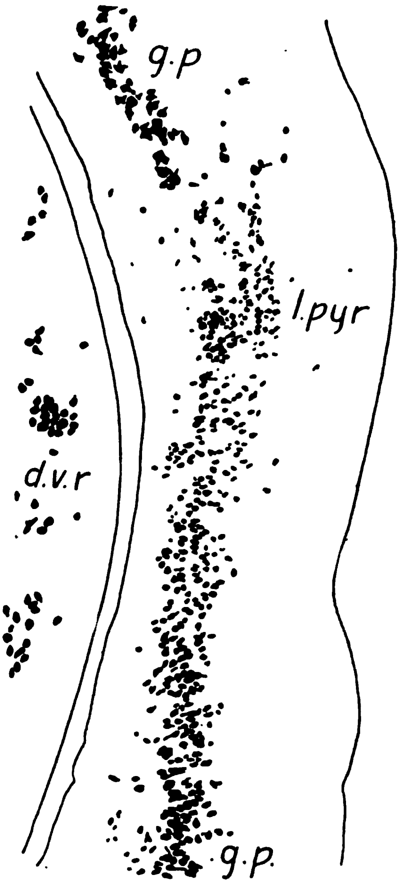
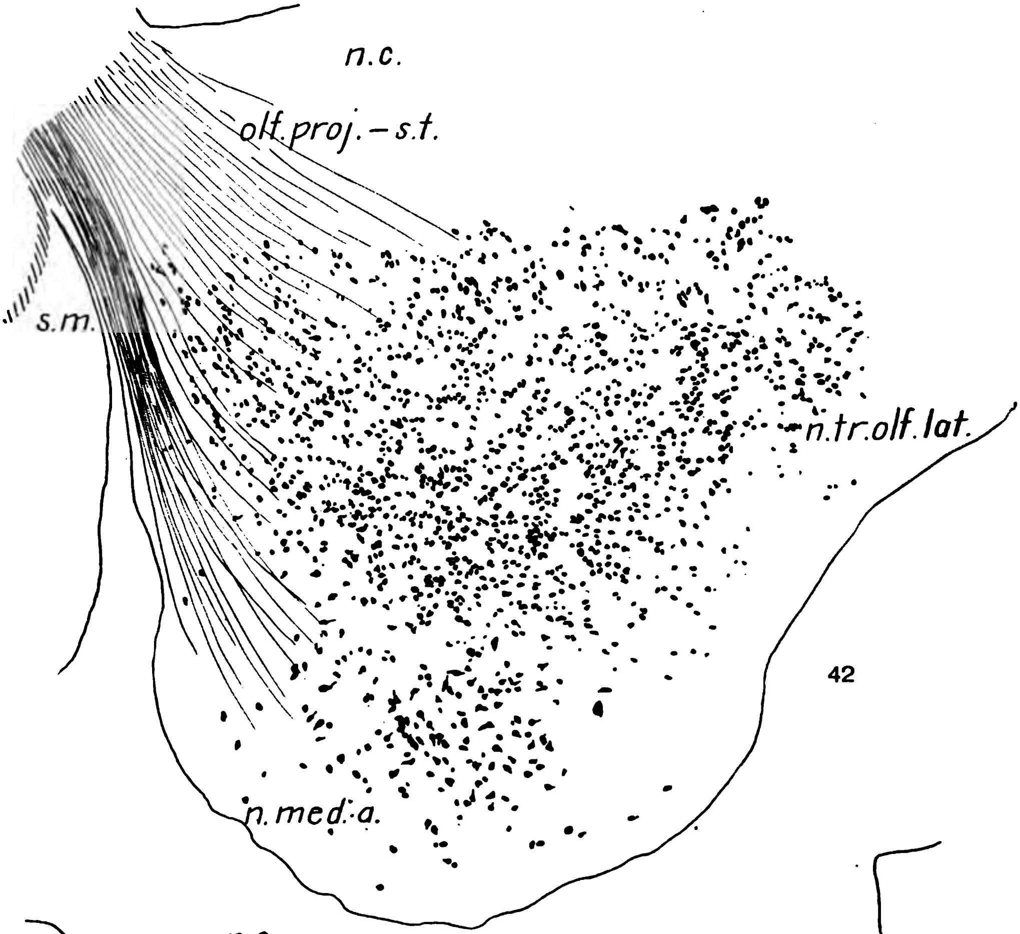


Fig. 41 Transverse section of the caudal part of the pyriform lobe. From the same section as figure 13. Magnification 70 diameters. In the upper part of the figure is seen the sharp dividing line between pyriform lobe and general pallium. No well-marked line of division is seen below.

Figs. 42 to 45 Four transverse sections through the region of the medial nucleus of the amygdaloid complex. From the same sections as 16, 15, 14, 13 respectively. Magnification 70 diameters.



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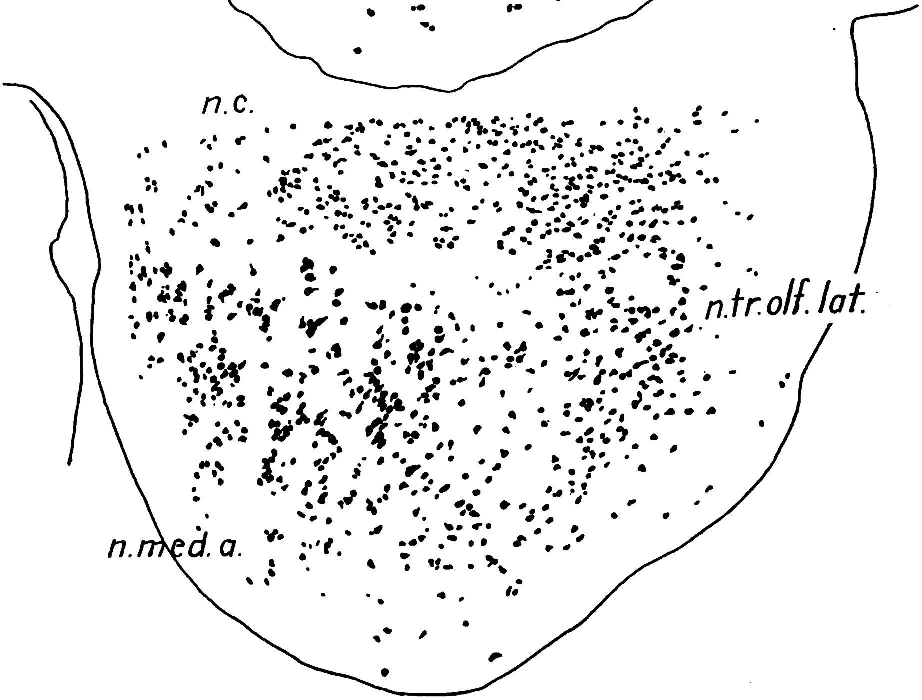

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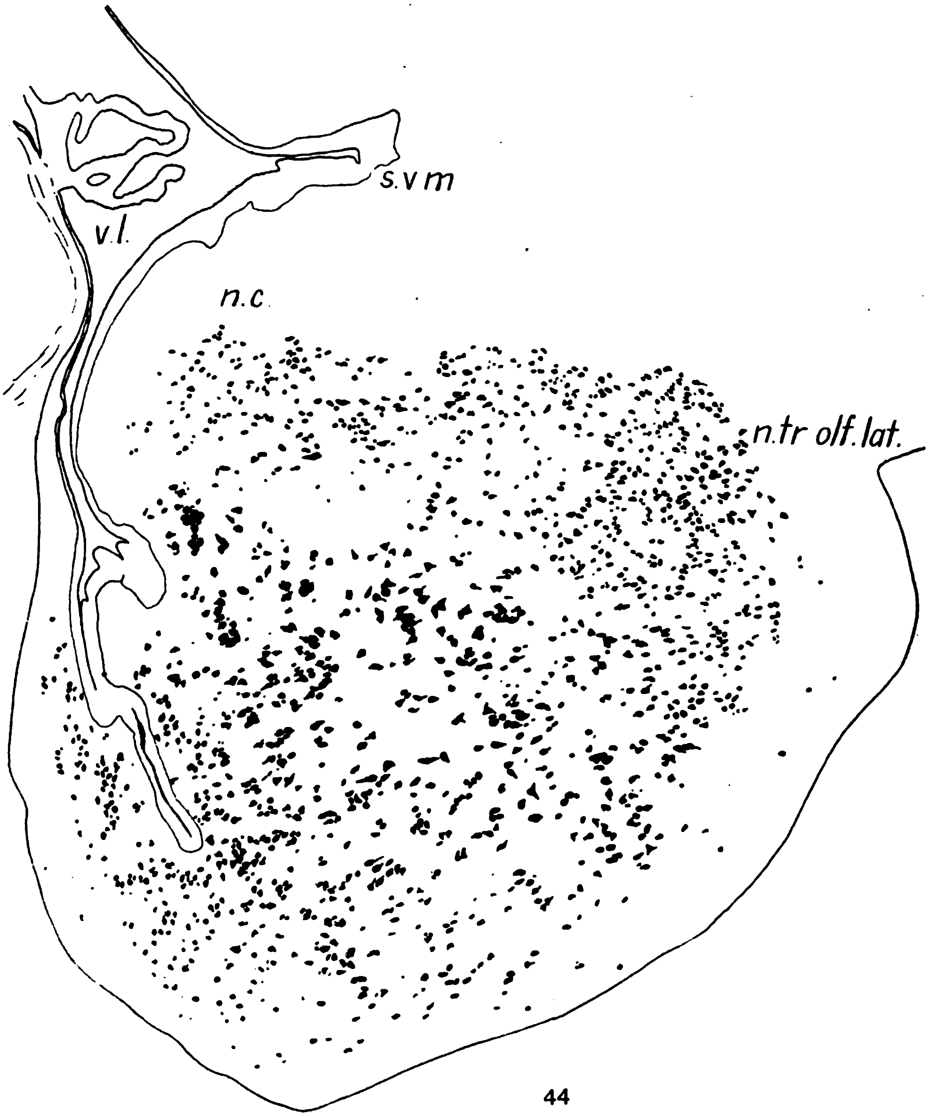

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Fig. 42 In this is seen the common mass of small cells formed by the caudate and the nucleus of the lateral olfactory tract. Below it is a mass of neuropile in which appear a considerable number of larger cells belonging to the medial nucleus. The stria medullaris enters into this neuropile while the olfactory projection tract and stria terminalis in part are related to the small-celled nucleus (compare fig. 49).

Fig. 43 Only a little of the small-celled mass is shown. The medial nucleus is larger and more dense and has shifted toward the medial wall.

Fig. 44 The medial nucleus is still larger and surrounds the temporal horn of the ventricle, the rostral end of which is cut by the section. Immediately caudal to this the medial wall of the ventricle is occupied by hippocampal formation.

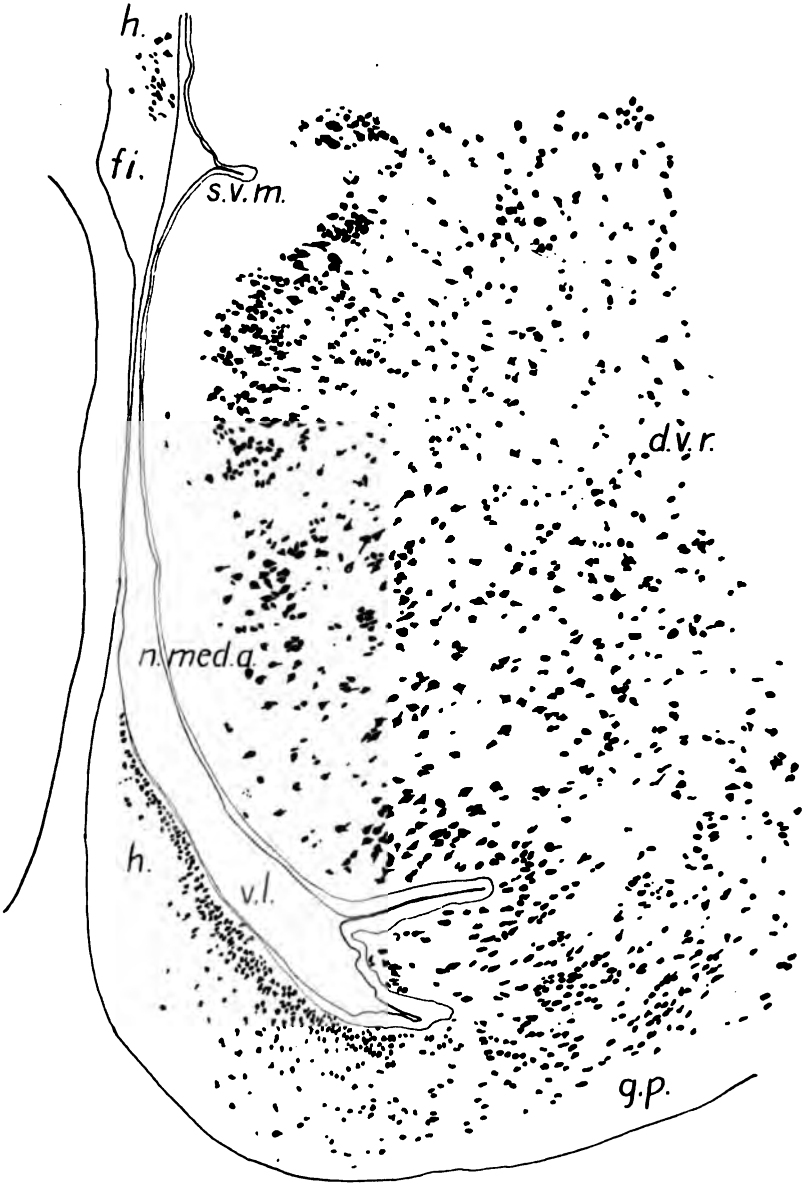


Fig. 45 In this section the medial nucleus occupies the ventricular surface of the basal lobe of the great ventricular ridge. Ventrally this mass of cells merges with the general pallium. The dense lamina in the medial wall belongs wholly or in part to the hippocampal formation. The small ridge in the lower angle of the ventricle lodges part of the stria terminalis bundle which spreads over the ventricular surface of the medial nucleus.

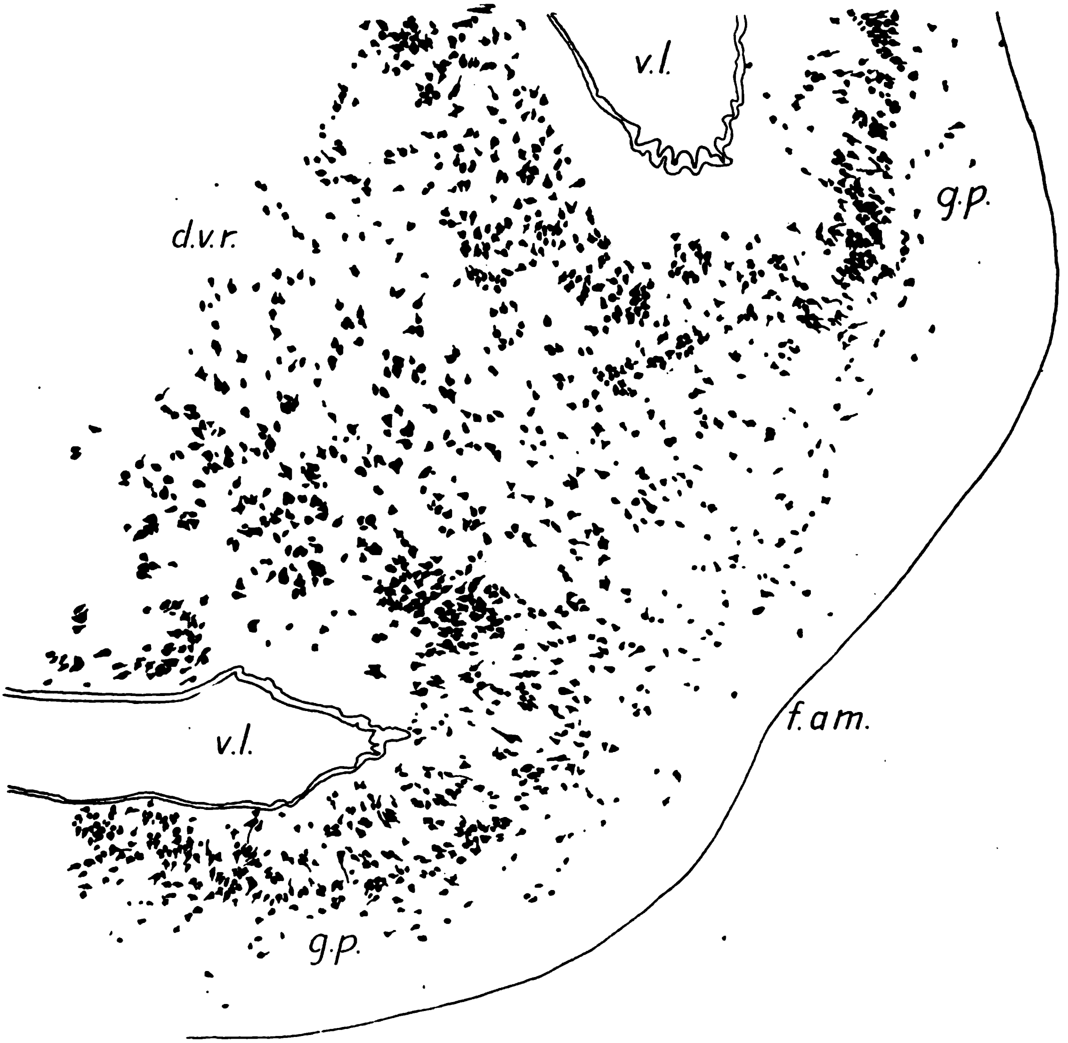
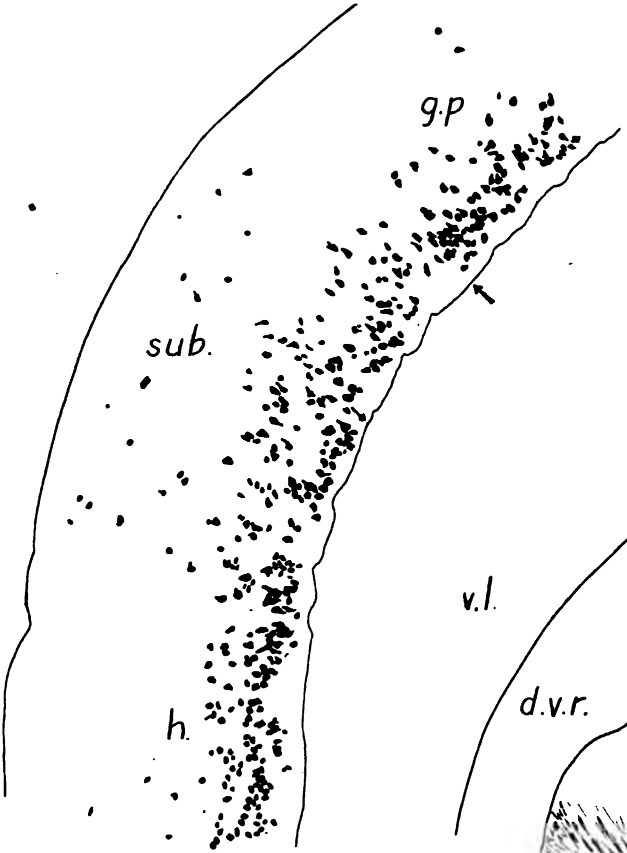


Fig. 46 Transverse section through the base of the ventricular ridge near its caudal end. From the same section as figure 11. Magnification 70 diameters. The relations are described in the text. The figure shows typical pallial walls laterally and ventrally which appear to be involuted to form the ventricular ridge.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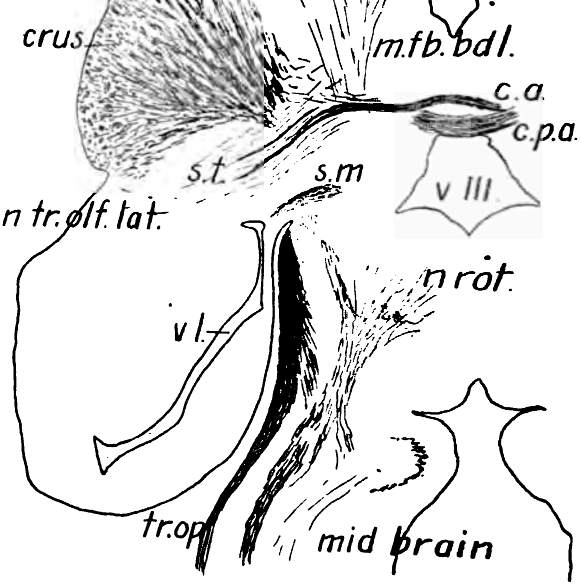
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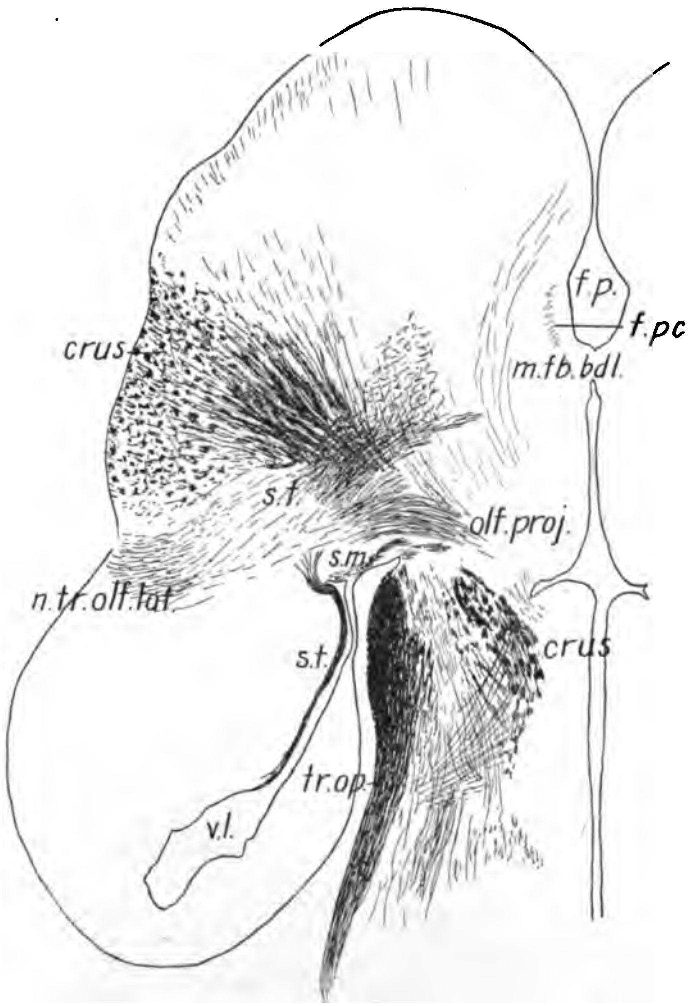

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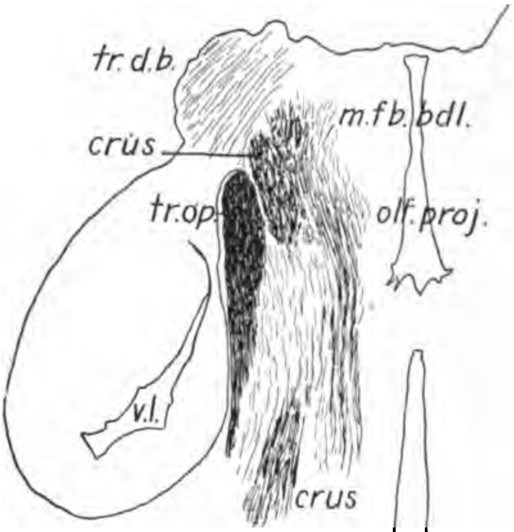
Fig. 47 A drawing of the subiculum region from the same section as figure 17. Magnification 70 diameters. The figure shows very inadequately the relatively dense layer of the hippocampus below, the more open arrangement of the cells in the subiculum and the abrupt medial border of the general pallium above. This border is marked by the arrow.

Fig. 48 Horizontal section from a Weigert series to show the anterior commissure and stria terminalis. The section is taken just at the level of the most dorsal part of the stria terminalis where it curves over the internal capsule. At this point the anterior commissure meets the stria (compare fig. 32) and continues with it into the small-celled nucleus of the amygdaloid complex.

Fig. 49 Horizontal section somewhat ventral to the level of that drawn in figure 48. This is from another series. The section passes through the lower or ventral part of the stria terminalis where it lies close upon and a little intermingled with the internal capsule. The section is drawn to show that the stria terminalis and the olfactory projection tract are divisions of a common broad bundle arising from the small-celled part of the amygdaloid complex. The bundle which enters the stria terminalis from the basal lobe of the ventricular ridge is drawn although it is almost wholly non-medullated. It can not be traced farther forward in these sections.



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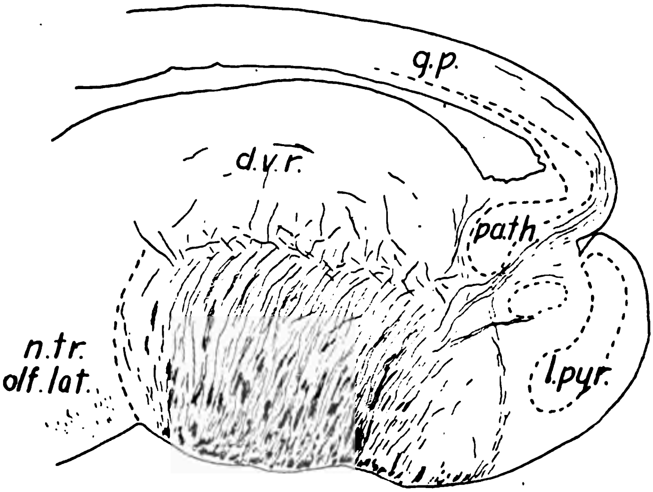

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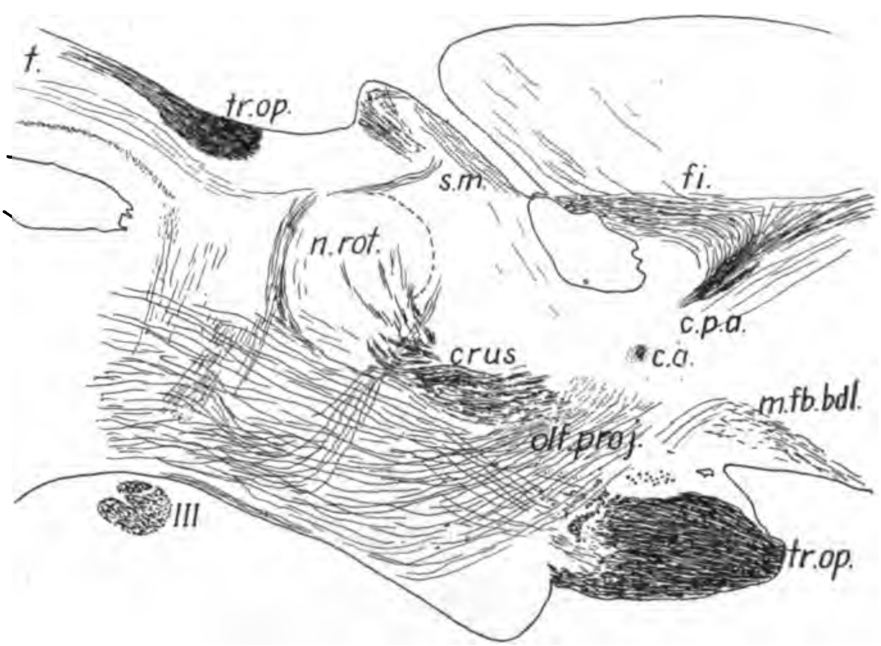
Fig. 50 Horizontal section through the temporal pole and thalamus. Same series as figure 49. This section shows the fiber bundle of the diagonal band where it crosses the basal surface of the crus and medial forebrain bundle.

Fig. 51 Parasagittal section close to the right lateral surface. Weigert stain. The cell masses of the pyriform lobe and pallial thickening are outlined in broken lines; also the line of division between the striatum and the amygdaloid complex. The figure shows especially the fibers of the internal capsule entering the pallial thickening.

Fig. 52 Section from the same series as the last. Near the median plane. The section shows the hippocampal commissure rising in the hippocampal primordium to join the fimbria system. There are shown also the relations of the sensory bundle of the crus, the olfactory projection tract and the medial forebrain bundle.



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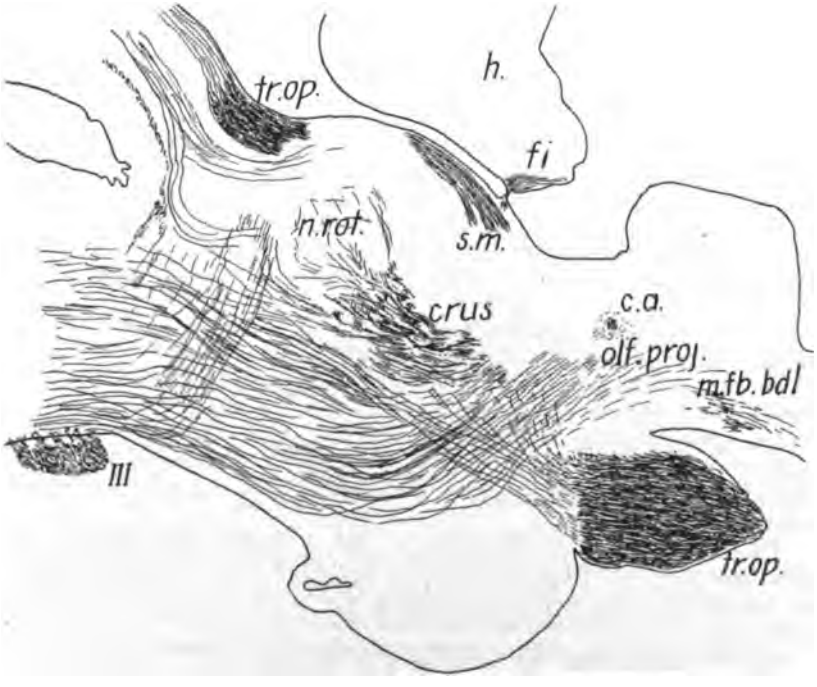


Fig. 53 A section from the same series as the last. It is taken from the left side of the brain. Owing to the oblique plane of the sections this is near the median plane at the level of the third nerve, but farther from the middle in the forebrain than in figure 52.

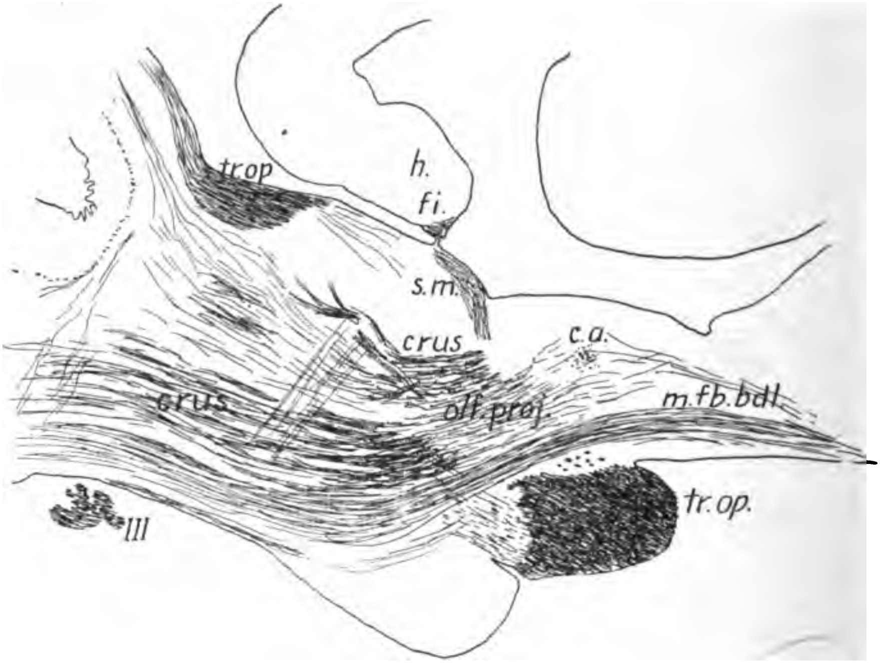
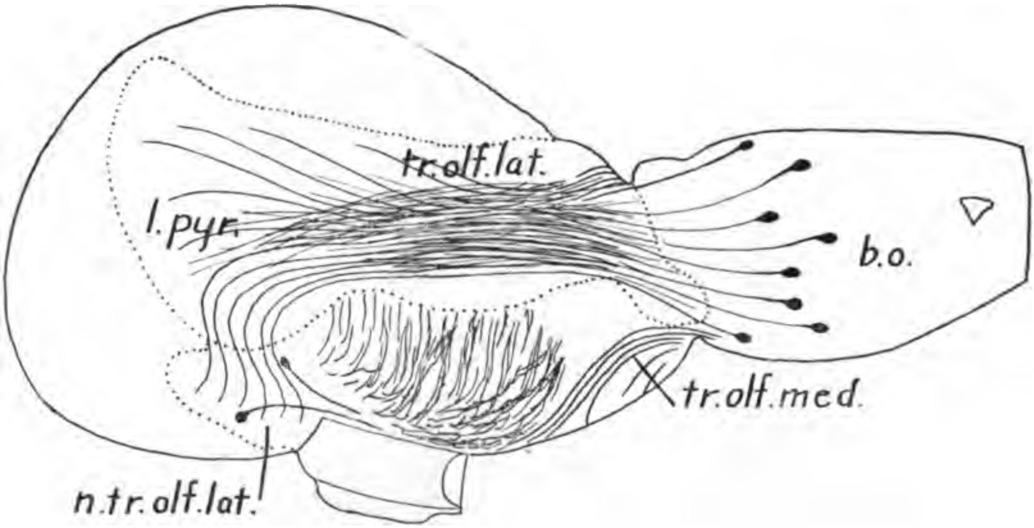


Fig. 54 A section from the same series, farther laterad than the last. This section shows a part of the sensory and the motor bundle of the crus, the olfactory projection tract and the median forebrain bundle. It is especially interesting to note from these sections that both the olfactory projection tract and the median forebrain bundle run almost wholly to the tegmental region or farther caudad, whereas it has been supposed that they both end for the most part in the hypothalamus.

Fig. 55 A diagram of the lateral olfactory tract together with a part of the medial olfactory tract and of the bundle of the diagonal band. Outline taken from figure 5. The figure shows the bundle which bends down behind the striatal area to end in the nucleus of the lateral olfactory tract in the amygdaloid eminence. At *a* is seen the bundle which comes from the medial wall of the olfactory bulb, crosses over the dorsal surface in the peduncular groove and joins the lateral tract. The whole course of the diagonal band can not be seen in this lateral view.

Fig. 56 A diagram of the olfactory tract in ventral view. Outline taken from figure 8. The medial and lateral bundles of the olfactory tract are separated by the striatal area containing the fibers of the crus. A bundle of the lateral tract curves around lateral and caudal to this area to reach the nucleus of the lateral olfactory tract, while a bundle of the medial tract passes along the ventral border of the striatal area to reach the same nucleus. From this nucleus, then, fibers enter the stria medullaris and the bundle of the diagonal band. The latter go to the hippocampus by way of the precommissural fornix system.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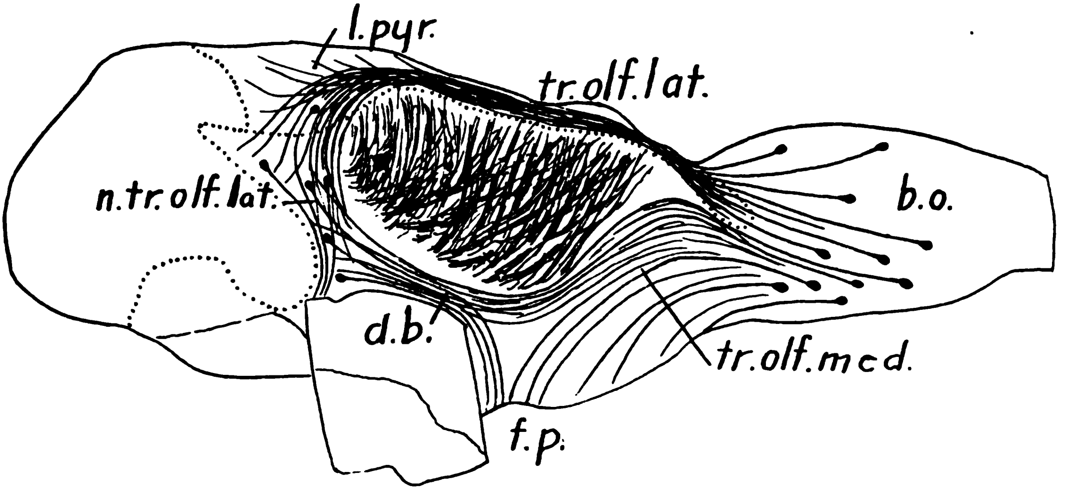
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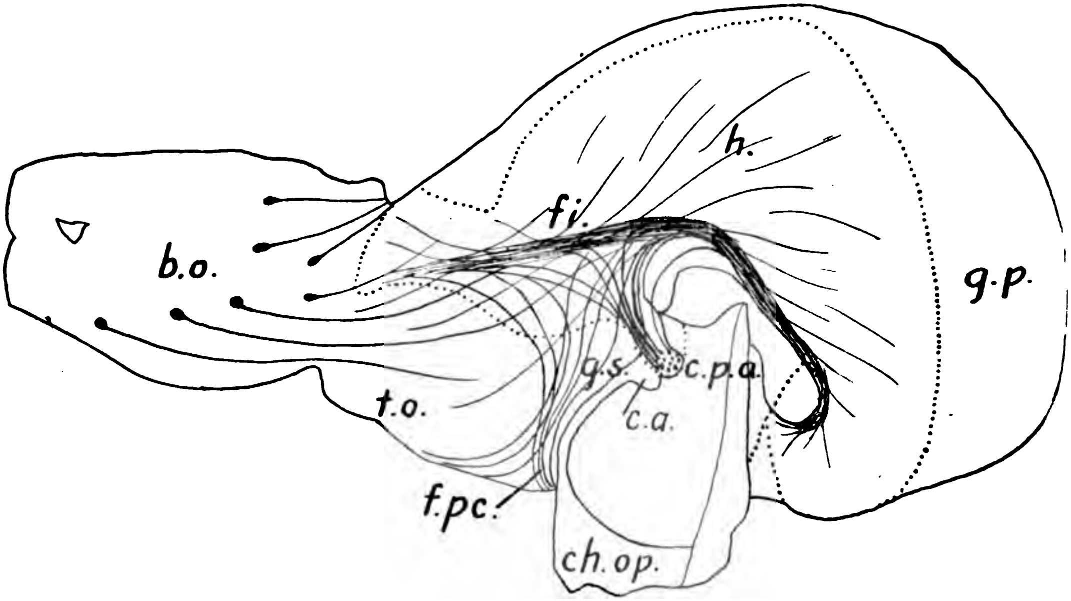

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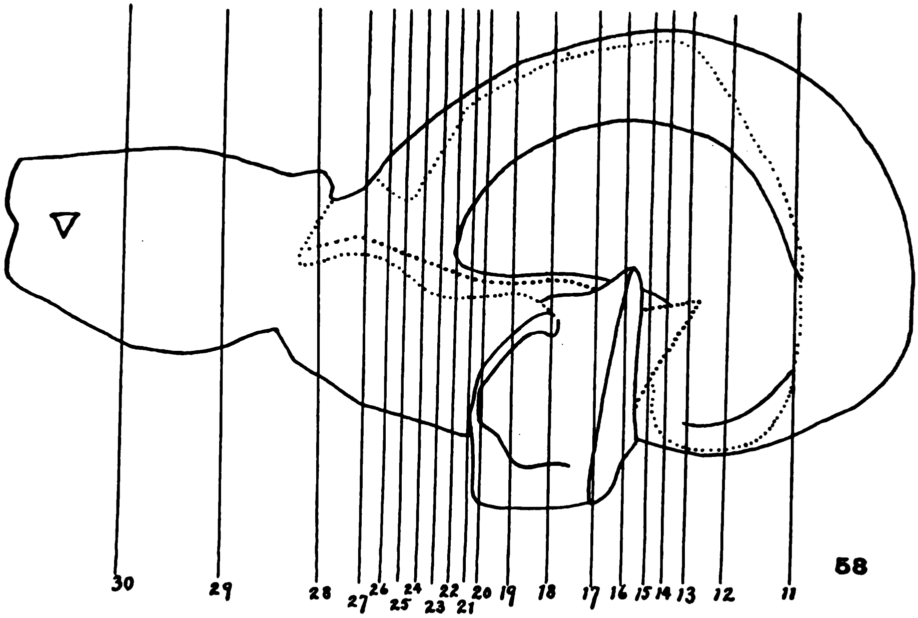

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Fig. 57 A diagram of tracts in the medial wall. Outline from figure 6. This shows especially how the fimbria system is made up of several components: olfactory tract fibers, precommissural fibers from the diagonal band, hippocampal commissure, and fornix (not drawn).

Fig. 58 Sketch of the right hemisphere after figure 10 with lines to show the position of the sections drawn in figures 11 to 30.

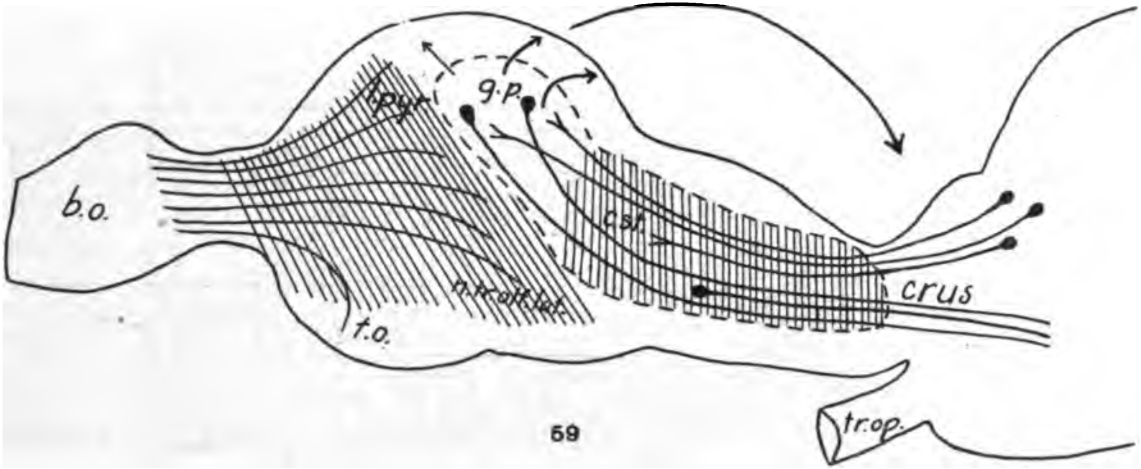

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Fig. 59 Schema of the selachian forebrain viewed from the left side. The outline corresponds to that of the forebrain in Scymnus or Heptanchus. The lateral olfactory area is shaded with oblique lines, the corpus striatum with vertical lines. The expansion of the somatic centers in the roof as general pallium is indicated by arrows and the direction of the chief expansion of the hemisphere is indicated by a large arrow. The equivalent of the pyriform lobe and the nucleus of the lateral olfactory tract lie rostral to the corpus striatum. The expansion of the pallium has been accompanied by a great elongation of this olfactory area.

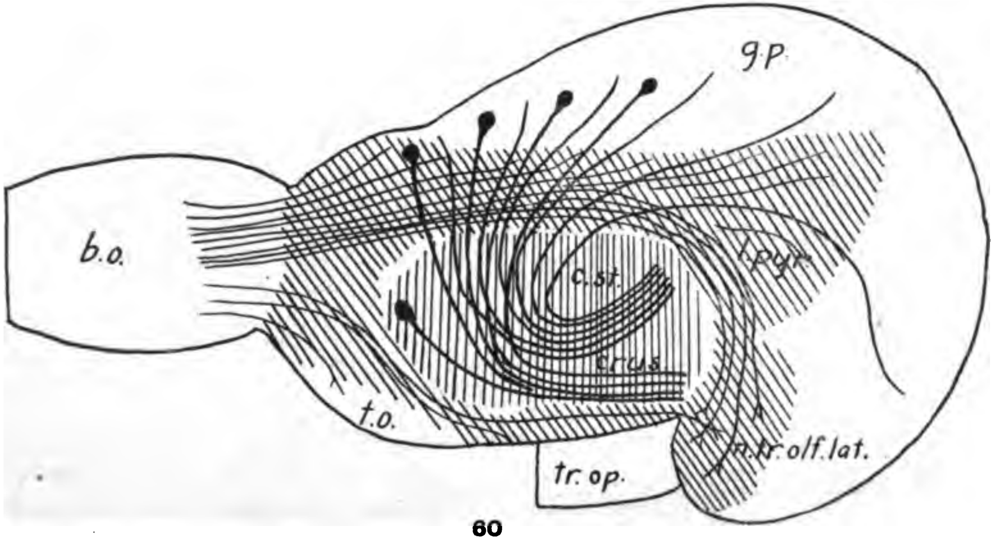

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Fig. 60 Schema of the forebrain of the turtle viewed from the left side, for comparison with figure 59. The olfactory area has been stretched over and beyond the corpus striatum but does not now form a covering of the striatum. The present condition in the turtle is such as would have been produced if the striatum had been covered by an olfactory layer and then the cells of that layer had withdrawn to the borders of the striatum so as to form a ring of olfactory centers around it.

Reprinted from The Anatomical Record, Vol. 9, No. 1, 1915

31. On the implantation and placentation in the Sciuroid rodents (lantern).

Thomas G. Lee, Institute of Anatomy, University of Minnesota.

In 1902 and 1903 the writer published descriptions of the implantation of the ovum in Spermophilus. In this work attention was called to a method of implantation and a series of structural changes preceding the formation of the true placenta which were unlike those of any other previously described mammal, and at the same time were the first account of the implantation in any of the Sciuroidae. These observations were confirmed on the European Spermophilus by Rejsek. In 1905 Müller found similar conditions in the European red squirrel, Sciurus. In 1910 the writer described the early stages of Cynomys at the International Anatomical Congress in Brussels. Since 1902 the writer has been engaged in collecting early stages of various genera of American Sciuroid rodents to determine if the peculiar conditions found in Spermophilus (or 'Citellus' as the taxonomists have since decided upon as the proper generic name) were characteristic of this large group of rodents. The collection of very early stages of wild rodents which breed for the most part but once a year, and whose genera are widely separated over the United States, is an extremely tedious and very expensive undertaking, but sufficient material has been secured to date of the following genera of the Sciuroidae to determine that the general method of implantation and placentation as previously described for Citellus (Spermophilus) hold true for the larger division. The genera studied include Citellus, 3 species, Ammospermophilus, Tamias, Cynomys, and Sciurus. The writer is preparing a more complete description and illustration of the early developmental conditions characteristic of these Sciruoidae than was possible before with the quite limited material. The greater variety of material now available enables one to point out the interesting slight divergences among the several genera.

Reprinted from THE ANATOMICAL RECORD, Vol. 9, No. 1, 1915

32. *On the relationship of the endocardium to entoderm in Citellus.* THOMAS G. LEE, Institute of Anatomy, University of Minnesota.

In studying the early development of the sciuroid rodent Citellus the writer noted the following described conditions which may be of interest to investigators working on that yet unsolved problem of the origin of the vascular system.

With the folding over and fusion of the entodermal walls of the foregut to form the pharynx region, there is to be noted a dorso-lateral angle on either side formed by the dorsal wall on either side of the chorda and the lateral wall of the pharynx and a somewhat less prominent ventro-lateral angle or groove, which will be designated in this paper as the cardiac sulcus.

Each cardiac sulcus is a groove or furrow in the free surface of the entoderm which follows the course of the lateral hearts. It begins in the lateral wall of the mid gut region and extends forwards to enter the closed pharynx region at the ventro-lateral angle and then continues along the ventral wall of the pharnyx converging to unite with the opposite sulcus in the mid ventral line just above the point of fusion of the lateral hearts.

The entoderm in the line of the cardiac sulcus is considerably thicker than that on either side. This thickening, however, is not uniform; it is more pronounced in certain areas than others. There is thus produced a corresponding elevation or ridge of the entoderm in the direction of the lateral heart. This thickened entoderm constitutes the walls of the sulcus. The groove, while easily recognizable throughout its course, varies in its shape; in places it is narrow and deep, in other places it becomes widened out and quite shallow. In embryos of this stage of development, the fold of splanchnic mesoderm which will form the myocardium does not completely envelope the endothelial tube of the lateral heart, the interval being completed by the entoderm of the foregut. It is this portion of the entoderm that forms the cardiac sulcus. The above described sulcus is not peculiar to Citellus but is figured by many investigators, as Kölliker in the rabbit, Fleischman in the cat, Bonnet in the dog. It is a transitory structure but is probably to be found in all mammals at the proper stage of development. While this region has been figured, almost no reference has been made to it as far as I am at present familiar with the literature.

In a number of series of Citellus I have found interesting examples of an intimate relationship between the entoderm of the cardiac sulcus and the endocardium of the lateral heart as shown by the reconstructions and drawings that illustrate this paper.

In the Citellus embryo here modelled, the primary implantation attachment (previously described by the writer) is just separating while the trophoblastic attachment for the allantoic placenta is beginning at the mesometrial portion of the uterine cavity; the amnion is not yet quite complete; the foregut is closed, the ectoderm of the oral plate is fused with the entoderm but not broken through; the pharynx does not yet show the evagination of the pouches; the endocardium of the lateral heart is beginning to fuse at the anterior end; the two dorsal aortae are well outlined, the first aortic arch is not yet completed. In an embryo at this stage the endocardium of the lateral heart on either side, in the region between the junctions of foregut and midgut and the point of beginning union of the two lateral hearts, shows an intimate relationship to the thickened entoderm of the cardiac sulcus. The endocardial tube is free and separate from the myocardial fold of splanchnic mesoderm in the sections, the contour of the tube is either oval or pear-shaped with a portion of the endothelial wall extended out as a thin fold or strand of cells toward the sulcus. Examining the series section by section it will be seen that while in each there is the extension of the fold or strand of cells toward the sulcus, in certain sections there is a short interval and in others very close contact; in certain sections there is distinct continuity with the entoderm of the sulcus walls. This intimate relationship is lost in the region of the fusion of the lateral heart.

Reprinted from THE ANATOMICAL RECORD, Vol. 8, No. 2
February, 1914

Proceedings of the American Association of Anatomists, thirtieth session, at the University of Pennsylvania, Philadelphia, Pa. December 31, 1913.

39a. The passage of the ovum through the uterine epithelium in Geomys bursarius, with demonstration of wax reconstructions. THOMAS G. LEE, Institute of Anatomy, University of Minnesota.

Within the order of Rodentia there exists a greater variety in the implantation of ovum and formation of decidual cavity than in any of the other main divisions of the Mammalia. The rage of variation extends from those forms, like the rabbit, in which the whole of the uterine lumen is utilized, to that of mouse and rat, where only a restricted portion of the uterine cavity is transformed into a decidual cavity. Then follows those peculiar forms, as Citellus (spermophilus), in which the writer in 1902 and 1903 described for the first time a rodent in which the trophoblastic layer of cells at close of segmentation caused the destruction of a small area of the uterine epithelium at the antimesometrial portion of uterine cavity, followed by an outgrowth of trophoblastic cells into the mucosa to form a nutrient organ, followed by the atrophy and disappearance of this organ upon the completion of the allantoic placenta at the mesometrial portion of the uterus; the whole of the uterine cavity being utilized for a decidual cavity, as in the rabbit. And lastly are found those forms in which, as in man, the ovum passes entirely through the uterine epithelium and a new decidual cavity is formed in the mucosa and independent of the uterine lumen. The first rodent of this type to be described was the guinea pig, so beautifully worked out by Graf Spee. In Geomys we find a second rodent of this type, but with the following important points of difference from the guinea-pig which are shown in these reconstructions. In the guinea-pig the ovum passes through the uterine epithelium at close of segmentation, and while it is very small in volume, the small perforation is quickly closed and the uterine cavity completely separated by epithelium from the new decidual cavity. In Geomys, on the contrary, the ovum perforates the uterine epithelium in the blastula stage, and when of so large a diameter that the edges of the large rounded perforation of the uterine epithelium cannot grow together as in the guinea-pig, or be filled with a fibrin plug as in man, this opening persists during the entire preplacental period. The epithelium at the lip of perforation is somewhat everted and gives a point of attachment to a zone of the trophoblastic layer of the blastocyst. The dorsal portion of the trophoblastic layer extends across the opening, and by this zonal attachment to the epithelial lip completely shuts off the uterine lumen from the new decidual cavity. The cells of the mucosa are broken down and the decidual cavity is rapidly enlarged. The blastocyst sinks down into this cavity but continues to be suspended by the above described zonal attachment to the lip of the epithelial perforation. This zone will ultimately form the outer margin of the allantoic placenta. A detailed description of the unique preplacental development in Geomys with plates will be published in the near future.

Reprinted from THE ANATOMICAL RECORD, VOL. 8, NO. 2
February, 1914

Proceedings of the American Association of Anatomists, thirtieth session, at the University of Pennsylvania, Philadelphia, Pa. December 31, 1913.

39b. An improved electric microscope lamp, with demonstration. THOMAS G. LEE, Institute of Anatomy, University of Minnesota.

This lamp was devised by the writer to meet the demand for a small compact and portable lamp for individual staff or student use in the Minnesota Institute of Anatomy, and has proved to be so satisfactory that it is here demonstrated for the benefit of the members of the Association. The lamp consists of a small vulcanite base fitted with a silvered reflector and a socket into which can be screwed as desired either a 2, 4 or 6 candle-power Mazda lamp with miniature base; a metal cap supported by 3 rods which fit into the base shuts off all side light and gives the necessary ventilation. In the top of this cap is an opening the same size as the base of the Abbe condensor. A device on the top of the metal cap holds in place over this opening the ordinary blue or ground glass plates commonly used with the Abbe condensor, thus giving at all times monochromatic light of uniform intensity. The base is fitted with flexible electric fixture wire terminating in a small plug which fits into a socket in the table top. The entire lamp is small, about two inches in diameter and in height, so that it readily fits in under the Abbe condensor when the mirror is pushed to one side. When not in use, the lamp can be put away in the student's locker with the rest of his outfit. A stock automobile Mazda bulb of standard make of 6 volts, 5 watts, alternating current is used in this lamp. It is the smallest available, the least expensive, and will withstand rough usage. In fitting up the laboratory tables a step down transformer is connected with the feed wire. This reduces the current used from 110 volts to 6 volts. These transformers are small, inexpensive, and can be had of any capacity desired, are used in the trade in sign lighting apparatus. The wires leading from transformer are run underneath the tables and connected to the sockets which are set into table top at any desired point. A detailed description of this apparatus with illustrations will appear in the near future.

Reprinted from The Anatomical Record, Vol. 9, No. 1, 1915

41. *Studies on the syrinx of Gallus domesticus.* J. A. Myers, Institute of Anatomy, University of Minnesota, Minneapolis.

The results of this work may be summarized as follows:

Structure. (1) The syrinx of the domestic chicken belongs to the tracheo-bronchialis type, and is quite simple when compared with the voice organ of song birds. (2) No intrinsic muscles are present in the syrinx of Gallus domesticus. The extrinsic paired sterno-trachealis with its caudal prolongations constitute the entire musculature of the syrinx. (3) The rigid skeleton is very highly modified. The first four tracheal rings are imperfectly fused to form the tympanum. The four intermediate syringeal cartilages are continuous ventrally with the ventral pyramid of the pessulus, while dorsally they end unattached. The first bronchial half-rings are large and in adults are attached and fused at both ends of the pessulus. The pessulus is the largest of all skeletal parts and lies dorso-ventrally at the junction of the bronchi in a plane transverse to the long axis of the trachea. The tracheal rings, the pessulus, and the ventral ends of the first half-rings become ossified, while all other skeletal parts remain cartilaginous. (4) The external tympanic membranes appear between the fourth intermediate syringeal cartilages and the first half-rings while the internal tympanic membranes extend from the caudal borders of the pessulus to the bronchidesmus and represent merely a modified part of the medial bronchial walls. (5) The syrinx is lined with stratified ciliated columnar epithelium containing numerous simple alveolar glands. Upon approaching the tympanic membranes this columnar epithelium is transformed into a stratified squamous epithelium which becomes a single layer of flattened cells over the membranes proper. (6) The tympanum is attached to the remainder of the syrinx only by elastic membranes.

Development. (1) The first indication of the respiratory system was observed in a 68 hour embryo in which the laryngeo-tracheal groove and the bronchi were represented. At first the trachea is much shorter than the bronchi, but with the development of the neck, it becomes, after the 140 hour stage, relatively much longer than the bronchi. The walls of the trachea and the bronchi are at first composed only of epithelium which contains two or three rows of nuclei. (2) The mesenchymal condensation common to the entire epithelial tube first becomes markedly prominent at the tracheal bifurcation in an embryo of 152 hours. (3) The anlagen of the first bronchial half-rings appear in a 176 hour embryo, those of the fourth intermediate syringeal cartilages appear 12 hours later. The anlagen of the third intermediate syringeal cartilages and the anlage of the pessulus are present at 200 hours. (4) Distinct cartilage cells were first observed in the first bronchial half-rings. (5) The first four tracheal rings have not united to form the tympanum at hatching, nor have the other skeletal elements taken the shape of those found in the adult. No bone is present at the time of hatching. (6) Ciliated cells are present in stages beyond 248 hours but were not observed in the region of the future tympanic membranes. (7) Mucous cells were first observed in 332 hour embryos and only in later stages were they found arranged in the form of simple alveolar glands. (8) Muscular tissue is differentiated in the 176 hour stage. Muscle fibers showing faint cross striations appear at 296 hours. At 452 hours the muscles are well developed. (9) At the time of hatching the tympanic membranes are thick. They are covered, however, as in the adult, with a single layer of epithelial cells.

Function. (1) That the syrinx is the true voice organ of the chicken is evident from the following deductions: First, structurally it is the only part of the respiratory tract capable of producing sound; Second, when the trachea is divided and the cephalic portion tightly tied off, the chicken is still able to crow; Third, after division of the trachea, voice can be reproduced artificially by forcing air into the air sacs. (2) The upper larynx serves only to modulate the voice. (3) The sterno-tracheal muscles by their contraction shorten the trachea and modify pitch. They also draw the tympanum cephalad, thus indirectly varying the tenseness of the tympanic membranes. (4) The air sacs are necessary in voice production, for voice could not be reproduced artificially after puncturing the cervical sacs.

Reprinted from The Anatomical Record, Vol. 8, No. 2
February, 1914

Proceedings of the American Association of Anatomists, thirtieth session, at the University of Pennsylvania, Philadelphia, Pa. December 31, 1913.

48. The development of the pancreas in selachians. Richard E. Scammon, Institute of Anatomy, University of Minnesota.

The pancreas of selachians is remarkable in that it arises from a single diverticulum which has generally been described as dorsal in position. It is, however, very difficult to determine the exact position of this anlage by the customary means of reconstruction. For this purpose, therefore, I have applied the method of graphic reconstruction devised by Weber. This method, which seems to me a most valuable device for studying the early stages of the larger glands, has apparently been employed only by its originator some ten years ago in an extensive study of the earliest stages of development of the liver and pancreas in Amniotes. The details of this procedure can hardly be given in the space allotted here. They may be found in Weber's original work (Arch. D'Anat. microsc., T. 5, '03), and will be again presented in this paper in its final form. In general terms, it is a modification of the graphic method of reconstruction in which the varying thickness of the epithelium of the reconstructed archenteron is represented in corresponding varying shades of color so that one may study the various areas of epithelial thickening in much the same way that one interprets the elevations of land in a topographic map.

A reconstruction made in this manner of the midgut of an Acanthias embryo 5.2 mm. in length, which is of a stage well preceding any indication of the pancreas as an outpouching, shows in the future pancreatic region of thickened epithelium which includes not only the dorsal zone of the gut but extends well ventrally. This pancreatic area is connected with a large thickening ventral and anterior to it which constitutes the liver anlage. These two thickenings, the pancreatic and hepatic, constitute, with the thickened epithelium connecting them, a ring which passes obliquely completely around the archenteron. The existence of such a pancreatic-hepatic ring was postulated long ago by Brachet and has been demonstrated to a great extent in Amniotes by Weber.

Reconstructions by Weber's method of the midgut regions of embryos of Acanthias 7.5, 9 and 10 mm. in length, show a gradual breaking up of this ventral hepatic segment. Between the two the epithelium remains somewhat thickened and in this occurs a particularly thickened patch which occupied the same position as does the anlage of the ventral pancreas in the higher vertebrates. This thickened plate never produces an outpouching and disappears in older embryos. While the dorsal outpouching of the pancreas is a single one, there is in Acanthias at least, an early indication of division into right and left lobes. This division is not very distinct and can hardly be demonstrated without Weberian and plastic reconstructions. In Acanthias the left lobe lies anterior to the right and is the smaller. It forms in embryos from 15 to 35 mm. in length a distinct antero-ventral mass which later is to a considerable extent incorporated in the descending limb of the gland. In such other selachians as I have studied this lobe lies posterior to the right one. This is probably because the left lobe is not differentiated until the gland begins to separate from the gut and in all selachians except Acanthias separation takes place antero-posteriorly. I do not think that the bilobed dorsal pancreas is to be regarded as a primitive condition. Rather it is produced by the clockwise rotation of the gut and the formation of the spiral valve. Bilobed dorsal pancreas anlagen are found in mammals and in selachians but have not been clearly demonstrated elsewhere unless one accepts the observations of von Küppfer the value of which has been rendered doubtful by the work of Piper and Nicolas. It seems then that the bilobed form of dorsal pancreas is limited to those forms in which the clockwise rotation of the gut (common to some extent to all vertebrates) takes place at an early period and that this form of pancreas is due to that rotation.

A more complete statement of the development of the selachian pancreas will be published in the near future.

Reprinted from **The Anatomical Record**, Vol. 9, No. 1, 1915

45. *The technique of Weber's method of reconstruction.* Richard E. Scammon, Institute of Anatomy, University of Minnesota. (Lantern slides.)

This method as used by its author was applied mainly to surfaces which were almost flat. Its extension to the study of rounded surfaces requires that a method of curvature elimination be adopted. In dealing with the surfaces of cylinders or cones this is easily done by correcting for vertical curvature alone, but where segments of spherical surfaces are involved corrections for horizontal curvature are also necessary. Vertical and horizontal corrections can be made so that the finished plat will give an approximately true representation of both the area and shape of a given outline upon a curved surface.

The method of making a finished reconstruction from the reconstruction plat has been considerably simplified by the introduction of color by means of 'Herring' papers and by building up the final reconstruction in strata.

The entire process involves the following steps: (a) Correcting section drawings for vertical curvature; (b) Scaling drawings for thickness; (c) Laying out reconstruction plat with corrections for vertical and horizontal curvature; (d) Plotting gauge lines; (e) Building up the finished reconstruction from the plat.

Reprinted from THE ANATOMICAL RECORD, Vol. 9, No. 3
March, 1915

ON WEBER'S METHOD OF RECONSTRUCTION AND ITS APPLICATION TO CURVED SURFACES

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Institute of Anatomy, University of Minnesota

FIVE FIGURES

In embryological work it is sometimes a matter of importance to determine with accuracy areas of epithelial thickening which are not demonstrated satisfactorily by the ordinary methods of graphic or plastic reconstruction. Placodal thickenings of the skin ectoderm, thickened zones in the neural tube, and the thickened areas found in the early archenteron are examples of structures which are not well demonstrated by these customary methods. A method of reconstruction which brings out graphically the extent and comparative thickness of such areas was devised some years ago by A. Weber, and was used by him with much success in the study of the very early development of the great glands of the digestive tract. Weber first published an account of his method in 1902,[1] and again a shorter summary in connection with the final report on his work in the following year.[2] Apparently the method has not been employed elsewhere. I have found it of such interest in the study of the earlier stages of the pancreas and liver that it has seemed desirable to present it here with some modifications which may be found useful, particularly in its application to curved surfaces.

Weber's method is based upon that of graphic reconstruction from transverse sections. An outline (from either lateral or dorsal view, as desired) of the organ to be reconstructed is plotted out on transverse section lines in the customary manner. Instead, however, of completing the reconstruction by indicating the *contour* of the surface thus outlined (as is commonly done), the *thickness* of the wall which forms that surface is measured in the transverse sections, and the variations in this thickness are plotted out upon the reconstruction. The reconstruction will then bear a number of lines which mark the boundaries between areas of epithelium of different thicknesses. To finish the reconstruction, the areas thus outlined are filled in with different shades of a single color, the darker shades being used for the thicker areas of the epithelial wall. The finished reconstruction then will exhibit the outline of the structure and the variation in the thickness of the part of its wall which is shown in this particular view. It will give no conception of the surface modeling of this wall aside from what can be determined from the outline alone.

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[1] Une méthode de reconstruction graphique d'épaisseurs et quelques-unes de ses applications à l'embryologie. Bibliog. Anat., T. 11.

[2] L'origine des glandes annexes de l'intestine moyen chez les vertébrés. Arch. d'Anat. Micr., T. 10.

The picture obtained is much the same as would be secured were it possible to remove the wall of the structure, render it translucent and, magnifying it greatly, hold it before a bright light. The thicker portions of the wall would then appear to the observer as darker areas, as they do in the reconstruction.

Figure 1 is a graphic reconstruction of the left side of the archenteron of an embryo of Torpedo ocellata, 4.0 mm. in length (No. 765 of the Harvard Embryological Collection). The portion of the gut lying on either side of the anterior intestinal portal and included between the lines *A* and *B* has been reconstructed by Weber's method and is shown in figure 4. The latter figure shows by means of its coloring a broad band of thickened epithelium extending dorso-ventrally across the gut at the level of the anterior intestinal portal. The lower and thickest part of this band represents the anlage of the gall bladder and liver, while the upper part includes the future pancreatic region. A thickened spur extends backward from this zone, and marks off the line along which the intestine will eventually separate from the yolk-stalk ventral to it.

The method of preparation of such reconstructions can best be explained in detail by following an example of the process.

For this purpose I will use the reconstruction shown in figure 4 which has just been described.

In preparing this reconstruction, drawings were made of each section of the portion of the gut involved, although fairly satisfactory results can be secured with drawings of every other section. These drawings should be made at a high magnification, preferably over 300 diameters. As the sections are drawn, they show of course the curvatures of the contour of the archenteron wall. It is desirable to eliminate these curvatures in the reconstruction and to present the gut wall as an approximately flat plane. If this is not done, areas which project sharply from the general plane of the archenteron will be represented in the reconstruction as much narrower than they actually are in the specimen or, if small, may be lost entirely. To eliminate these curvatures, it is necessary to divide the outer margin of each section into a number of short cords or segments, each of which will be comparatively straight, and to lay off segments of an equal length on the transverse section lines of the reconstruction. This can be done with a pair of small screw compasses. For work at a magnification of 300 diameters and over,

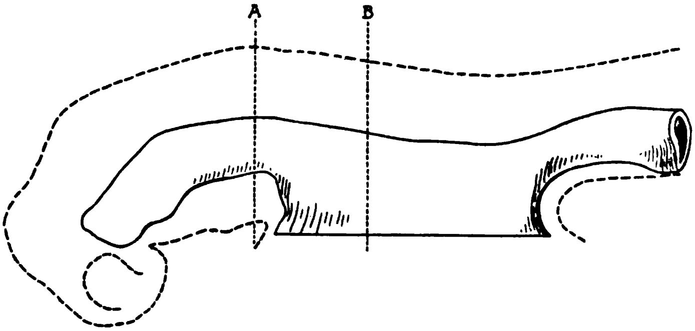

Fig. 1 A graphic reconstruction (lateral view) of a portion of the archenteron of an embryo of Torpedo ocellata 4.0 mm. long (H.E.C. 765). × 30. The outline of the embryo is represented in broken line. The archenteron is drawn in solid line. The portion of the archenteron lying between the dotted vertical lines *A* and *B* is shown in a Weber's reconstruction, at higher magnification, in figure 4.

segments of 1 cm. are small enough to eliminate most of the error from curvature. Figure 2 is of a section (No. 104) of the reconstruction under discussion. The short lines on the inner side of its margin represent the boundaries of these centimeter segments.

The thickness of the epithelium forming the gut wall is now to be measured in each drawing. For this purpose a unit of measurement must first be determined. Working with drawings made at a magnification of 300, it has been found that the 1.5 mm. is the smallest unit practicable for such a scale. By using this unit one is able to measure variations of less than 5 micra in the thickness of the gut wall, and errors of projection and drawing would probably render more refined measurements of little value. A scale of 1.5 mm. units is laid out upon a stiff card, or better, a piece of transparent celluloid. This scale or gauge is then passed over each section, care being taken to keep its graduated margin at right angles to the *axis* of each segment of the drawing and its zero point at the inner margin of the epithelium. This process is begun at the dorsal median line on each section and is carried laterally or ventrally from that point. At the first place where the thickness of the epithelium is found to correspond to a graduation point on the scale, a fine line is drawn out to the side of the section and the thickness noted at its end. The scale is carried along the section until a point is reached where the thickness of the epithelium corresponds with the next graduation (either above or below the former one) on the scale. Again a line is drawn out from the section at this point and the thickness noted. This process is continued until the entire side of the section has been gauged and marked off into segments in which the variation in thickness is not over 1.5 mm. on the drawing or 5 micra in the corresponding section. An example of a section thus gauged is shown in figure 2. As shown by the gauge lines, the epithelium immediately below the dorsal median line is between 30 and 25 micra (6.5 mm. at × 300) in thickness. The thickness falls to 25 micra at the point indicated. This is followed by a broad zone which is less than 25, but over 20 micra thick. Ventral to this zone, the epithelium increases to a thickness of between 35 and 40 micra, and then again decreases to less than 10 micra as it approaches the blastoderm. The arrows seen in the figure point towards the thinner edge of the segment and are used to avoid confusion in mapping out the gauge lines on the reconstruction at a later time. It is important that in gauging the section drawing the scale be held at right angles to the long axis of that particular part of the wall which is being measured rather than at a similar angle to either the inner or outer surface of the epithelial strip. This holds particularly when gauging the thickness of an epithelial band which

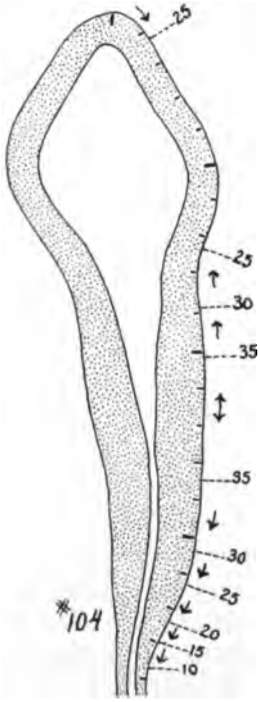

Fig. 2 Drawing of a transverse section of the archenteron of a Torpedo embryo 4.0 mm. long (H.E.C. 765) showing the method of measuring sections for reconstruction by Weber's method. The short lines extending into the section mark the centimeter segments used in eliminating lateral curvature from the section. The longer lines extending out to the right from the section are the gauge lines. The figures at the end of the gauge lines indicate in micra the thickness of the epithelium at the points touched by them.

is rapidly changing in caliber, or when gauging a portion of a band which forms a sharp curve. Failure to observe this precaution causes noticeable error both in the gauge readings and in the position of the gauge points on the drawing. There will often be encountered considerable portions of the wall, the thickness of which corresponds exactly to the gauge unit or a multiple of it. My rule, made arbitrarily, has been to mark as the gauge point the first (i.e., most dorsal) level at which such a zone is encountered.

A reconstruction outline is now made on section-lined paper in the usual manner, except that only the dorsal margin of the gut is mapped out. Using this margin line as a base, the transverse section lines are divided into centimeter segments corresponding to those made on the margins of the drawings of the sections. In practice it is convenient to mark the point separating each block of 5 cm. segments in a different color to aid in plotting. The ventral margin of the gut and the gauge points, which have been determined on the cross section drawings, are now plotted on the transverse lines of the reconstruction in the usual manner, except that instead of measuring the distance of each point from the dorsal margin of the section and transferring this measurement to the corresponding section line as is customary, one measures the distance of the point from the nearest centimeter mark in each case. The ventral outline of the reconstruction and the gauge lines indicating the thickness of the epithelium are established by connecting all the points of the same order as is done in an ordinary graphic reconstruction. The reconstruction will now have the form seen in figure 3. There the base and transverse reconstruction lines are lightly drawn and the centimeter segment points are indicated as small dots on the latter. The outline of the reconstruction is indicated in heavy line. The ventral margin posterior to the anterior intestinal portal has been cut away arbitrarily in a straight line. The gauge lines indicating the thickness of the epithelial wall of the organ are represented in heavy broken line. The vertical figures placed at the termination of the gauge lines indicate their values in micra.

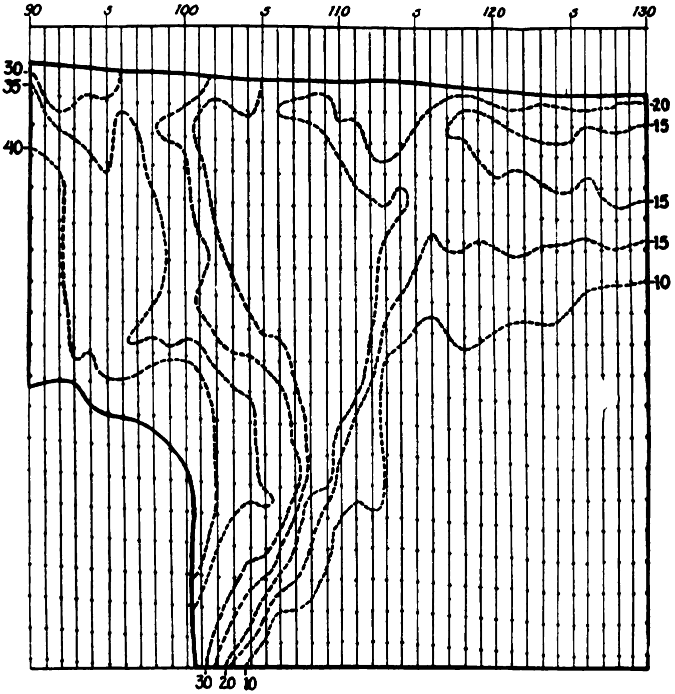

Fig. 3 Reconstruction plat of a Weber's reconstruction (lateral view) of the portion of the archenteron bounded by the lines *A* and *B* in figure 1. The light vertical lines represent the transverse sections. The outline of the reconstruct on is represented in heavy solid lines. The gauge lines bounding areas of epithelium of different thicknesses are represented in heavy broken lines. The dots on the transverse lines represent the boundaries of the centimeter segments described in the text and shown in figure 2. The vertical figures at the margins of the reconstruction give the value of the gauge lines in micra. The figure is reduced to one-half the size of the original reconstruction, which was made at a magnification of 350.

There remains but to color in the areas which are marked off by the gauge lines. Weber did this with water-color and secured excellent results, as his figures show, but a much simpler and quite as satisfactory a method is to use papers of different shades of gray for the different areas. Such papers should be as near 'pure' mixtures of black and white as it is possible to secure. The only kind which I have found satisfactory is the 'Herring' series which has been prepared for color work in psychological laboratories. The shades numbered 3, 4, 7, 10, 16, 20, 30, 42 and 46 make a satisfactory series of marked but fairly equal gradations.

To build up the final colored reconstruction gray papers are selected, equal in number to the areas mapped off on the reconstruction by the gauge lines. The entire outline of the reconstruction is now traced upon the lightest colored paper. This area is cut out and pasted firmly upon a piece of compo or plaster board. Upon the paper of the next darker shade there is traced an outline similar to the preceding except that the space representing the thinnest area is cut away. This second sheet is pasted upon the first one so that the similar angles and sides correspond. In this way the area of thinnest epithelium is represented by the lighter colored paper and all thicker areas by the darker one. This process is continued, all thinner areas being cut away from each new outline until the darkest and final shade of paper will have the shape and will represent the area of the thickest epithelium only. This method of building up the papers of different colors in strata will be found much easier than to cut out each separately and then attempt to fit them together in a mosaic. Figure 4 is a half-tone made directly from such a colored reconstruction and based upon the plotting illustrated in figure 3.

The example just described is of a lateral view reconstruction. Reconstructions may be made by Weber's method to show dorsal or ventral views of epithelial structures as well. For this purpose the transverse sections of the structure are drawn and measured in the same manner as that described. A reconstruction plat is then laid out as follows. A vertical line is drawn in the middle of the sheet to represent the dorsal median line of the structure and transverse section lines are drawn on either side at the proper distance apart and at right angles to the median vertical one. The centimeter points, which on lateral view reconstructions must be measured off on each transverse section line, can be located on the plat in this case by simply ruling vertical lines parallel to and at centimeter intervals from the median one. The gauge points and lines marking the boundaries

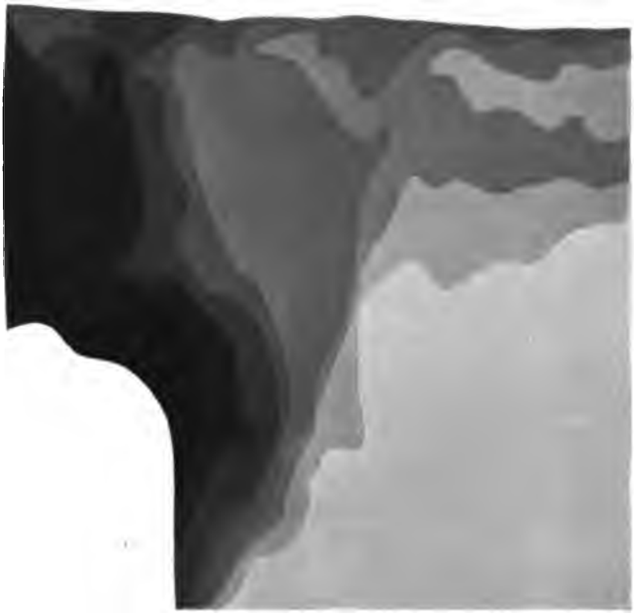

Fig. 4 A finished Weber's reconstruction (lateral view) made from the plat represented in figure 3 and including that part of the archenteron lying between the lines *A* and *B* in figure 1. The thicknesses of the epithelium in several parts of the reconstruction are represented by the various shades of gray. Starting with the lightest, these several shades represent the following epithelial thicknesses; (1) below 10μ; (2) 10 to 15μ; (3) 15 to 20μ; (4) 20 to 25μ; (5) 25 to 30μ; (6) 30 to 35μ; (7) 35 to 40μ; (8) above 40μ. This figure is reduced to three-fifths the size of the original reconstruction.

between areas of different epithelial thickness are mapped out as in the lateral-view reconstruction. Should the reconstruction be of a tubular structure, the ventral median line must be determined in each cross section drawing. In reconstructing, the tube is then represented as split along its ventral median line and flattened out laterally; i.e., the lateral margins of the reconstruction represent in fact the ventral median line of the original tube. Figure 5 is an example of such a reconstruction plat made from the same object employed for the lateral view reconstruction already described. The method of representation and lettering are the same as used for figure 3.

Several of the possible uses and advantages of this method of reconstruction have been mentioned. It remains to speak of a few disadvantages and sources of error. In the first place the outlines of the reconstructions, aside from the one used as a base line, are not strictly such as would be secured by the ordinary graphic or plastic methods. In eliminating the lateral curvatures from the transverse sections, the dimensions of the figure are increased dorso-ventrally or laterally, as the case may be, without a similar increase antero-posteriorly. Weber made no attempt to eliminate the curvature seen in cross sections, regarding it in fact as of some value in indicating contour. I have already pointed out the disadvantage in reconstructing without making this correction, which I think should be done even at the expense of some accuracy of outline, which can easily be determined by the other reconstruction methods.

While the error introduced by this method is small when dealing with structures having surfaces approximating those of cones or cylinders, it is considerable when applied to spherical surfaces. Spherical surfaces do not admit of being spread out into planes as do those of cones and cylinders. Cartographers, who have much this same problem in representing large areas of the globe, have developed a number of methods of projection to meet, in part, this difficulty. These are, however, too complex for our work and are all based upon representations of the perfect sphere. For the purpose at hand surfaces which approximate spherical ones can best be treated by first compensating for the vertical curvature by the method of segmenting the outline of the section already described; and, second, by allowing for longitudinal or horizontal curvature by increasing the distance separating the cross section lines on the reconstruction plat. The latter can be done by determining the actual length of the outline of the structure to be reconstructed and dividing this distance by the number of sections which the structure contains. Multiplying the figure thus secured by the magnification at which the reconstruction outlines are drawn gives one the distance which should separate the section lines on the reconstruction plat. This practice differs from that of ordinary reconstruction in

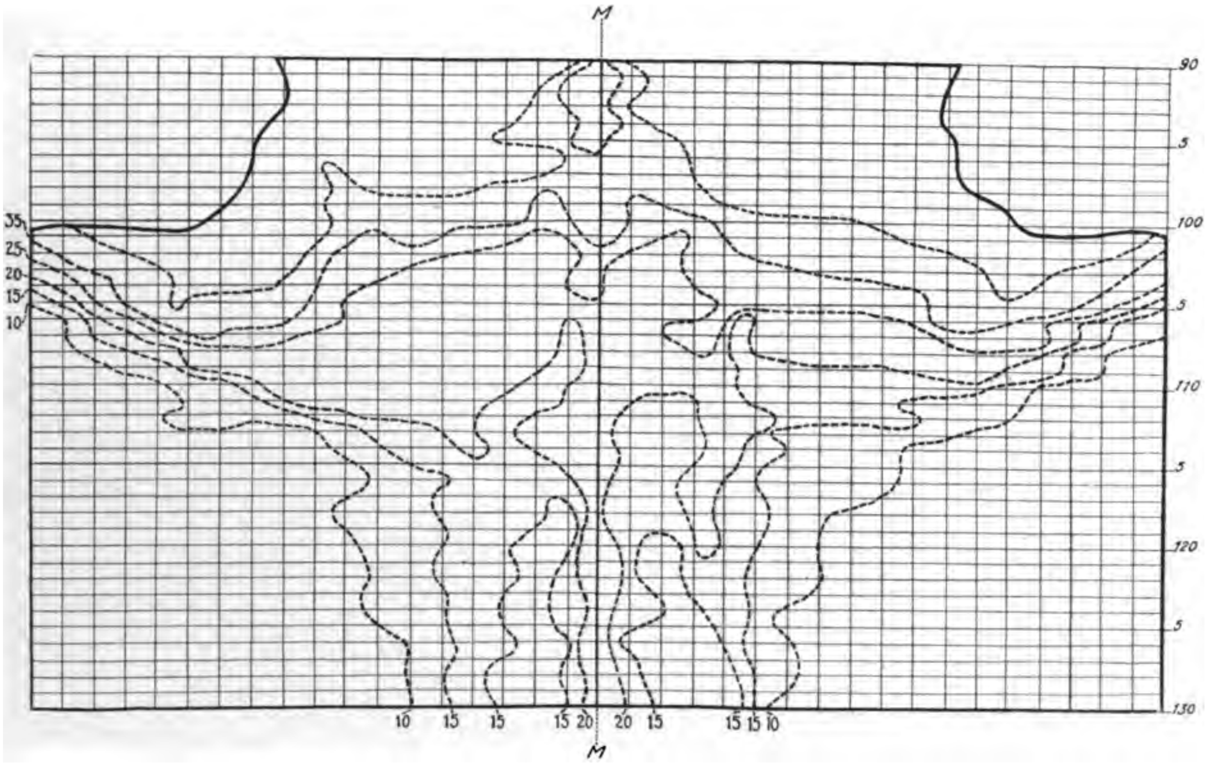

Fig. 5 Reconstruction plat of a dorsal view reconstruction of the same object as shown in figures 1, 3 and 4. The line *M-M* represents the dorsal median line of the archenteron. The lighter lines parallel to it are the boundaries of the centimeter spaces. Scheme of drawing and lettering corresponds to that of figure 3. The scale of magnification is the same as for figure 3.

that the longitudinal axis of the structure is used in the customary method instead of the actual length of the outline as in this case. Reconstructions made with these corrections will show approximately the area and shape of any given outline upon a curved surface, although neither will be strictly accurate. As a rule such correction will be unnceessary unless one is dealing with surfaces which curve very abruptly. Measurements of the thickness of epithelial plates which curve longitudinally or horizontally will always be a little exaggerated because such plates are cut somewhat obliquely by transverse sections. There seems to be no practical way of eliminating this error.

Finally, the reconstruction will represent the variations in thickness of the epithelium as occurring in definite steps and not as gradual transitions as in nature. Most of this artificial distinction can be eliminated by using the smallest unit of measurement practicable and thus increasing the number of shades present in the reconstruction while decreasing the degree of their difference. Great reduction of figures in their reproduction also aids in securing the effect of gradual transition.

Reprinted from THE AMERICAN JOURNAL OF ANATOMY, VOL. 17, NO, 3
March, 1915

THE HISTOGENESIS OF THE SELACHIAN LIVER

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FORTY-FIVE FIGURES (SEVEN PLATES)

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INTRODUCTION

The structure of the adult selachian liver is far removed from that type which is generally considered characteristic of the organ in vertebrates. This variance from the common vertebrate type is seen in the great accumulation of fat in the hepatic cells, in the comparatively slight development of the bile duct system and in the absence of lobulation of the kind generally found in higher vertebrates.[1] However, these pecularities which distinguish the selachian liver are not manifested until a comparatively late stage in the development of the organ. In earlier stages characters common to the liver in all vertebrates, but which are often masked or modified in higher forms, are shown with unusual clearness. It is chiefly with these more fundamental characters such as the formation and anastomoses of the hepatic cylinders, the differentiation of the minor bile ducts and the relation of the parenchymatous and vascular structures in the liver, that this paper has to do. The specific characters of the selachian liver, which have been mentioned, have been considered only incidentally.

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[1] The histology of the adult elasmobranch liver was first briefly described by Leydig ('51) from observations on Chimaera. He published a more complete account dealing with several forms of selachians in 1853. Later descriptions of the general histology of the adult liver have been given by Shore and Jones ('89), Pilliet ('90), and Holm ('97). Deflandre ('05) has investigated the fat content of the hepatic cells, and Monti ('98) has studied the bile capillaries by the Golgi method.

The main material employed in this study consisted of embryos of Squalus acanthias, but specimens of Raia batis, Torpedo ocellata, Mustelus canis, Mustelus laevis and Squatina angelus have been used for supplemental and comparative work. For a large part of the Acanthias material, and for the specimens of Mustelus laevis, I am particularly indebted to the late Dr. Charles S. Minot, who not only permitted the removal of numerous series from the Harvard Embryological Collection, but also provided special material for use in this work.

DEVELOPMENT OF THE HEPATIC CYLINDERS

1. *Literature*

Our conception of the glandular structure of the vertebrate liver rests upon a large number of observations made mainly in the first half of the last century and culminating in the work of Eberth ('66) and Herring ('72). Since that time it has been recognized that the liver is a compound gland with a more or less regularly branching system of ducts and with a terminal network of anastomosing end pieces, and our knowledge of the details of this network has been greatly extended by the Golgi method in the hands of Retzius ('92), Hendrickson ('96) and others.

It is generally stated that the anastomotic type of liver is a modification of the compound branching gland type. Of this the best proof is the phylogenetic one, for in the lower cyclostomes, the liver is indeed a true compound branching gland, as has been shown by the work of Retzius ('92), Holm ('97), Cole ('13) and others. In Petromyzon, however, anastomoses occur occasionally in the embryo (Brachet '97) and are very numerous in the adult (Renaut '97). On the side of embryology little evidence of this modification has been offered, although the statement that the liver arises in many vertebrate embryos as a branching gland, and that it takes on its adult reticular structure through the anastomoses of its end pieces, is common enough in texts. But an examination of the literature shows no more complete account of this early period of the histogenesis of the liver than that given by Remak ('55) excepting Hilton's work ('03) on the pig which has hardly received the attention that it deserves. The study of the actual course of the anastomosis of the end pieces has also been neglected, and the only clear statement to be found regarding this process is not in connection with the liver at all but in Laguesse's ('96) description of the histogenesis of the pancreas where he found numerous anastomoses at different stages.

The literature of the histogenesis of the liver in selachians is scanty and has been to a large extent reviewed in the comprehensive works of Oppel ('00) and Fiessinger ('11).

Balfour's ('76) account was the first to appear and as it has formed the basis of all later descriptions, it is given here in full:

> By stage K the hepatic diverticula have begun to bud out a number of small hollow knobs. These rapidly increase in length and number and form the so-called hepatic cylinders. They anastomose and unite together so that by stage L there is constructed a regular network. As the cylinders increase in length their lumen becomes very small but appears never to vanish.

Hammar ('93) in the course of a study devoted particularly to the development of the larger hepatic structures illustrates the development of the hepatic cylinders in two models of the liver of Torpedo embryos corresponding in development to Balfour's stages L and M. He makes no comment upon them aside from stating that the trabeculae increase in number and decrease in caliber in the period intervening between the two stages.

Brachet ('95) studying Torpedo embryos determined with accuracy the area of the hepatic diverticulum which gives rise to the hepatic tubules, and confirmed Balfour's description of the structure of the hepatic cylinders in later stages.

Holm ('97) figured and described very briefly sections of the liver of two Scyllium embryos of advanced stages.

Most of our later information concerning the embryonic hepatic parenchyma comes from the studies of Braus ('96) on embryos of Acanthias, Spinax and Scyllium. Confirming Balfour's observations in regard to early stages he noted a complete and regular anastomosis of the tubules in Acanthias embryos of 38 mm. Here the tubules were of even size and consisted in cross section of seven cells surrounding a lumen of variable size but distinctly larger than that seen in the adult. The hepatic cells of this stage were free from fat. In older embryos of Spinax and Acanthias the cells were fat laden. Braus saw no side branches nor blind endings of any hepatic capillaries.

Choronshitzky ('00), studying Torpedo, found several secondary hollow outgrowths from the hepatic pouch in his "Stadium II," which corresponds approximately to Balfour's stage K, and to the Normal-plate Nos. 22–24. In "Stadium III" which is represented by considerably older embryos, the liver pouches give rise to a number of small hollow buds, the cavities of which communicate with that of the pouch. "Die Leber macht im allgemeinen den Eindruck einer verzweigten Drüse." By "Stadium IV" the hollow buds have been transformed into much branched hepatic trabeculae which contain no traces of lumina. Choronshitzky's opinion of the mechanical influence of the blood vessels on the formation of the hepatic tubules will be discussed in a later part of this paper.

Minot ('00) in discussing the development of sinusoids, mentions the presence of the first short hepatic cylinders in an Acanthias embryo of 11.5 mm. and speaks of their anastomosis and growth in older stages. He noted the interesting retardment of development which is to be seen in later stages in the caudal tip of the liver as compared with the cardiac end.

Debeyre ('09) made use of observations upon the development of the hepatic cylinders in Acanthias to lay the ghost of the theory of the mesodermal origin of the hepatic parenchyma, which had been again raised a short time before by Géraudel ('07). He gives no complete or detailed history of the cylinders but notes, with illustrations, their general appearance in embryos 16, 22, and 30 mm. in length, respectively. In the latter he recognized the beginnning of a period of pronounced increase in the diameter of the cylinders. Debeyre noted the presence of numerous granules in the apices of the hepatic cells and bases upon this the interesting suggestion that the liver may serve as an organ of internal secretion during a part of embryonic life.

2. Early development of the hepatic tubules

In this account the structures which have been variously termed hepatic cords, trabeculae, cylinders and tubules will be spoken of as tubules as long as they remain as portions of simple or branching unanastomosed glands. The term hepatic cylinders will be employed for the same structures after the process of anastomosis has taken place.

The exact time when the anlagen of the hepatic tubules first make their appearance is somewhat variable. In general they are first to be seen in embryos from 7.5 to 9 mm. in length, being somewhat younger than Balfour's stage K[2] and corresponding to numbers 22 and 23 of the Normal plate series. Such embryos have from fifty to sixty-five segments and four or five pairs of gill pouches of which the anterior three or four may open externally. The spiral valve is in the process of formation, making at this time one or two complete turns of the intestine and the vitelline duct is reduced to a short wide canal. The form of liver anlage at this stage is represented somewhat diagrammatically in figure 1. The organ consists of a ventral median pouch from the foregut just anterior to the vitelline duct. The anterior part of this pouch is full and rounded and may be termed the pars hepatica mediana. From the median pouch spring two large lateral pouches which form together the pars hepatica lateralis. In stages just preceding the appearance of the tubule anlagen the lateral pouches are smooth and globose and project outward almost at right angles to the median hepatic pouch. At the time when the tubules are formed, however, the lateral pouches are flattened transversely and have entered upon a pronounced dorsal growth. Connected with the liver pouch above and in front and with the anterior wall of the yolk stalk behind is a small thick walled sac, the anlage of the gall bladder. The hepatic tubules take origin entirely from the pars hepatica mediana and the pars hepatica lateralis. These areas are indicated in stipple in figure 1.

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[2] In correlating embryos with stages of Balfour's series only the general development of the embryo has been considered and not the state of development of the organ under discussion.

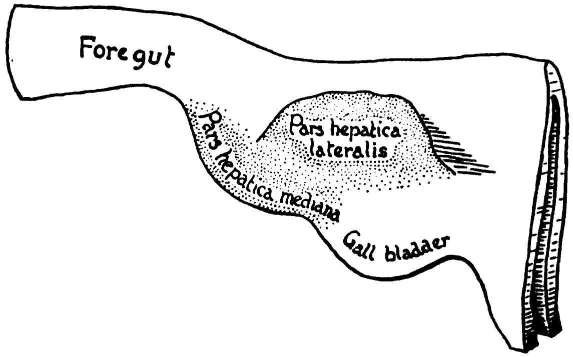

Fig. 1 Lateral view of a semi-diagrammatic reconstruction of the foregut and liver of an Acanthias embryo 9 mm. long. The areas represented in stipple give rise to hepatic tubules.

The tubule anlagen arise in two forms: as slight longitudinal ridges upon the lower part of the outer surface of the lateral pouches and as very small irregularities of the dorsal margins of the same structures. When first observed the longitudinal ridges are two to four in number on either side. They extend almost the entire length of the lateral pouches and are distinctly separated by shallow lateral furrows. Sometimes these ridges may be subdivided longitudinally at their ends. Figure 17 is a view from the left side and below of the liver of an embryo 7.5

mm. in length (S.C. 14).[3] This specimen bears two ridges on the left hepatic pouch, but the right pouch is entirely smooth. The irregularities of the margin of the hepatic pouches, which also form tubules, are at first so ill defined as to be scarcely noticeable unless reconstructed. Then it is seen that the formerly straight dorsal margin has a wavy contour.

The tubule ridges rapidly increase both in size and number. New ridges appear ventral and mesial to the earlier ones and extend to the base of the lateral pouches and upon the ventral surface of the pars hepatica medialis of the median liver pouch. No new ridges appear dorsal to the first ones and I think that no new ridges arise between older ones. By the time the embryo reaches a length of 10 to 12 mm. each lateral pouch wall and lateral half of the pars hepatica medialis bears seven to ten tubule ridges. This is the total number formed on either side, and when tubules thereafter arise from the pouch directly, they do so as individual tubules and not in the form of tubule ridges. Figure 18 is a view from the left side and below of a reconstruction of the liver of an Acanthias embryo 10 mm. in length (S.C. 20) showing the later form of the tubule ridges and the beginning of the differentiation of tubules from them.

Before all the tubule ridges are formed the ones which first develop are broken up by transverse or oblique furrows into rather irregular rows of low mound like elevations. These elevations are the anlagen of the individual hepatic tubules and may be seen in figure 18 referred to above. They are semicircular or nearly so in cross section and nearly twice as long as broad, their greater length being always directed antero-posteriorly. Almost immediately the tubules begin an active outward growth and each is differentiated into a distal extremity which often is large and pouch like, and a more slender proximal stalk which is connected with the hepatic pouch and which is circular in cross section. The further growth of the tubules takes place by the formation of tubules of the second order from the distal expansions of the primary ones. The secondary tubules are short conical projections rarely over one and one-half times as long as their greatest diameter. They arise from the sides of the distal expansion and almost always extend out at right angles to the axis of the primary tubule. Those primary tubules which arise from the sides of the hepatic pouches and are packed in among their fellows commonly assume a T-shaped form with the limbs of the T directed antero-posteriorly. The primary tubules which arise from the margins of the hepatic pouches become somewhat larger than those formed from the tubule ridges and give rise to from three to seven secondary tubules from their expanded distal chambers. These secondary branches may again subdivide into branches of the third order and upon these in turn there may occasionally be found nipple-like projections which represent the fourth order of tubules. In the large majority of cases, however, anastomosis takes place before tubules of the fourth or even the third order are formed. Figures 19 and 20 are of wax reconstructions of tubules from the lateral hepatic pouch of an embryo 13.3 mm. in length (S. C. No. 18). In figure 19 one sees the beginning of the outpouching of the distal expansion into secondary tubules. These structures are well marked projections in the older tubule shown in figure 20. Figures 22 and 23 are two views of a hepatic tubule from the dorsal margin of the left hepatic pouch of an embryo 15 mm. long (H.E.C. 227). The latter figure shows tubules of the fourth order. Figure 21 is a wax reconstruction of two young primary tubules from the ventral surface of the pars hepatica medialis of the same 15 mm. embryo. One of these shows the beginning of tubules of the second order.

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[3] In designating embryos the following abbreviations will be employed: H.E.C., Harvard Embryological Collection; K.U.E.C., Embryological Collection, Department of Zoology, University of Kansas; S.C., author's collection.

When the tubule ridges first appear on the hepatic pouches they are due to the increased thickness of the epithelium in these places and not to an evagination of the pouch wall. In cross sections of the ridges (see fig. 28, a cross section of the lateral wall of the left hepatic pouch of an embryo of 8 mm. K.U.E.C. 542) it is noticeable that the nuclei which in other parts of the wall are arranged in two interlocking rows tend to be reduced to a single row which lies near the external surface of the epithelium. There seems to be but a single layer of very high columnar cells at this point but the cell boundaries are not very clear. There is always a broad clear zone of cytoplasm towards the lumen of the pouch opposite the tubule ridge. Mitotic figures do not occur in the ridges but are frequent in the zones of epithelium between them. Following the formation of the ridge there appears a very shallow trench on the internal surface of the epithelium. The lumina of the individual tubules appear as the tubules themselves are differentiated through the breaking up of the ridges into rounded anlagen. One then finds in each tubule anlage a narrow slit-like cleft passing between the cell walls at right angles to the pouch cavity. This is shown in figure 29. Thus at first the tubule cells do not lie at right angles to the tubule lumen but nearly parallel to it. Later the cells assume an oblique position (see again fig. 29) and finally come to lie in the typical radiating position in relation to the lumen (fig. 30). As the tubules develop the cells become shorter and their nuclei change from elongately ovoid to nearly spherical bodies.

3. *The anastomosis of the hepatic tubules*

The liver is transformed from a gland of the branching to one of the reticular type by the anastomosis of its end pieces. The process begins in Acanthias in embryos from 12 to 15 mm. in length. Such embryos have from 70 to 85 pairs of somites and correspond roughly with Balfour's stages L and K and with Nos. 25–26 of the Normal plate series. The tubules arising from the dorsal portions of the lateral liver pouches precede in their differentiation those of other regions and it is generally among these dorsally placed tubules that anastomoses are first found. Later the tubules of the lower parts of the lateral pouches and finally those of the pars hepatica medialis enter upon the process. Variants from this general plan of procedure are not uncommon. Figure 2, of a sagittal section of the left hepatic pouch of an embryo 14 mm. long (S.C. 30) shows how general anastomoses are when once they begin in a given region.

No particular degree of differentiation seems necessary before a tubule takes part in an anastomosis, and when this process begins in any region both branched and simple tubules fuse indifferently. The commonest form of anastomosis is that established by the end to end fusion of tubules, but tubules may also join end to side or side to side. The last type is much commoner in later stages when more opportunities are offered for this form of contact. Anastomoses also seem to take place with equal frequency between tubules formerly quite separate or between minor branches of the same tubule when it is possible for such branches to come in contact.

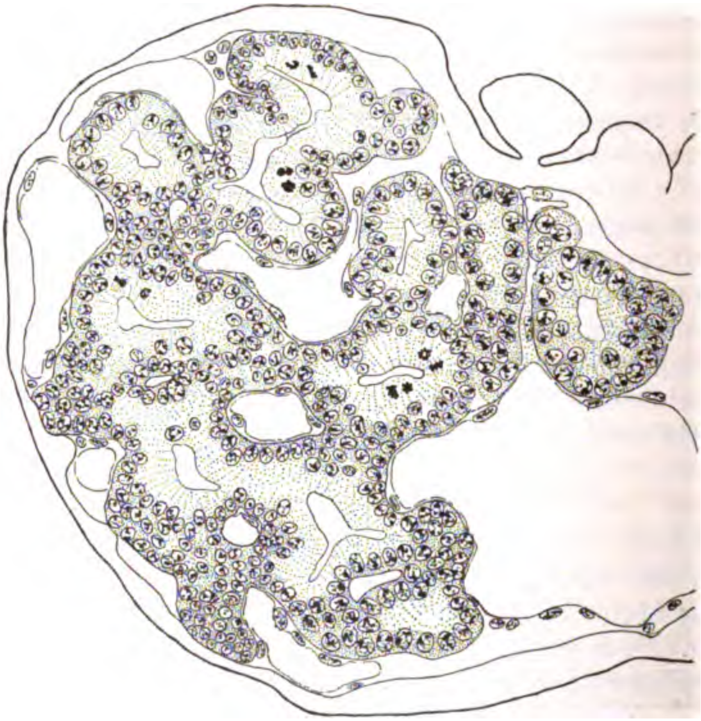

Fig. 2 Sagittal section of the anastomosing tubules of the left hepatic pouch of an Acanthias embryo 14 mm. long (S.C. 30.) × 100.

Figure 24 is of a wax reconstruction of three anastomosing tubules from the left hepatic pouch of an embryo 15 mm. in length (H.E.C. 227). The pouch wall from which the tubules spring is cut away squarely around the base of each tubule. Tubules *A* and *B* are completely anastomosed end to end but there is still a distinct constriction at the plane where they have joined and the lumen within is considerably narrower than in the bodies of the tubules. Although not clearly shown in the figure both *A* and *B* are T-shaped and in each case the other tip of the tubule ends freely without anastomosis. Tubule *C* is a compound one and the subdivision which passes over to join with *B* is a branch of the third order. There is as yet no actual anastomosis between *C* and *B*, but the epithelial walls of the two are in direct contact.

The lack of any account of the histology of anastomosis seems to warrant a rather full description of the process here. The process may be rather arbitrarily divided into three steps or stages. In the first step the tubules come in actual contact; in the second there occurs the fusion of their walls and a rearrangement of the cells forming them, and in the third there is an establishment of a connecting channel between the two original lumina.

These stages of anastomosis can be observed in the liver of Acanthias embryos of any length from 13 to 45 mm. They are more easily followed, however, in young specimens. The figures which are used here to illustrate the process have been taken from embryos under 20 mm. in length. In each case the sections have been followed through and the tubules involved have been reconstructed to make sure that the picture presented was not due to an oblique plane of section or a misinterpretation of a segment of a single complicated tubule.

Early stages of contact of the anastomosing tubules are illustrated by figure 30 which is a transverse section of the left hepatic pouch of an embryo 13.3 mm. long (S.C. 18). Numerous tubules extend outward from the lateral wall of the hepatic pouch. Of these the larger number are still in the form of simple tubules which are expanded distally but a few have entered upon anastomosis. The blood vessels pass among the tubules covering them in part with a thin film of endothelium which is interrupted at some places by the attachment of strands of mesenchyma and at others by the apposition of the distal surfaces of tubules with the insheathing layer of splanchnic mesothelium. Whether this endothelial covering forms an absolutely continuous partition without fenestra between the blood and the tubule epithelium cannot be definitely determined without injected specimens, but there seem to be places where the separation is not complete. Anastomosis is inaugurated by the contact of the involved tubules. At first a few mesenchymal or endothelial cells may separate the tubules but these are apparently pressed to either side so that the entodermal cells soon lie in actual contact. Often at first only four or five tubule cells actually meet, but shortly there is formed an area of contact which generally is not quite so large as the caliber of either tubule involved. Sometimes the cells of one tubule indent the wall of the consort but this is not the common rule. There are no basement membranes about the hepatic tubules, but the basal margins of the cells seem a little thickened so that for a time after contact the line between the cells of the two anastomosing tubules is still distinct. With the disappearance of this line the tubules may be considered as fairly fused.

The connection between the two fused tubules is often drawn out a little forming a short stalk or bridge between them. This will be termed here the connecting stalk. An indentation upon the external surface of this stalk indicates the line of fusion of the tubules. The connecting stalk, when present, is generally a little less in diameter than are either of the tubules and the boundaries of the cells forming it are not clear. One can follow the cells, however, by the position of their nuclei. Although at first sight the nuclei appear scattered without order in the stalk or at the point of anastomosis, a little study enables one to determine with some accuracy which nuclei are contributed by each tubule. The two rows of nuclei approach one another and may interlock but their radial arrangement in regard to the lumina of the two original tubules is not lost at first. They then pass to one side or the other of the connecting stalk, leaving a clear cytoplasmic core in which cell boundaries are faintly distinguishable. In so doing the axes of the nuclei rotate through an angle of 90° so that instead of being parallel to the long axis of the connecting stalk as at first, they are now at right angles to it. Such rotation can be determined, of course, only in young tubules where the nuclei are oval and not circular in section. Figure 26, drawn from an embryo 19 mm. in length (S.C. 3), shows an early step in these changes. Figure 3 A is a graphic reconstruction of the tubules shown in cross section in figure 26. In this section the rows of nuclei belonging to the two tubules involved are distinguishable although the process of migration of the nuclei to the sides of the connecting piece is clearly under way. In figure 36 of a later stage from an embryo 14 mm. in length (S.C. 30), all the nuclei with the exception of one have passed to the sides of the connecting stalk.

In following the course of the nuclei in tubule anastomosis one is but tracing the movements of the cells in which they are contained, for it is hardly to be considered that the nuclei shift their axes within the cells, and moreover the few faint cell boundaries which may be made out show the same changes in position as do the nuclei. The hepatic cells of the connecting stalk have shifted through an arc of about 90° and when a lumen is established through the center of the connecting stalk it is bounded at least in greater part by the same cell surfaces which were presented to the lumina in the original tubules. In other words, while the tubule cells involved in anastomosis have shifted in position, their surfaces and their axes will bear the same relation to the new lumen which they did to the former one. Their long axes will be at right angles to the lumen while the inner and outer surfaces of the cell remain constant in both the original position in the simple tubule and the later position in the anastomotic segment. The polarity of the cell, in the sense of the term as used by Rabl ('88, '90), is not disturbed by anastomosis.

The lumen of the anastomosis is formed by clefts which extend out from the lumina of the formerly simple tubules. These clefts are at first small and irregular. They pass between the rather irregular borders of the radially arranged cells of the connecting stalk or plate, and, meeting, become confluent. It is not until some time after these clefts have joined that the lumen of the anastomosis acquires its full size and regularity. A late stage in the establishment of this connecting channel is shown in figure 27 from an embryo 19 mm. long (S.C. 3). Here the clefts are still separated by a single cell. Figure 3 B is a graphic reconstruction of the tubules involved in this anastomosis.

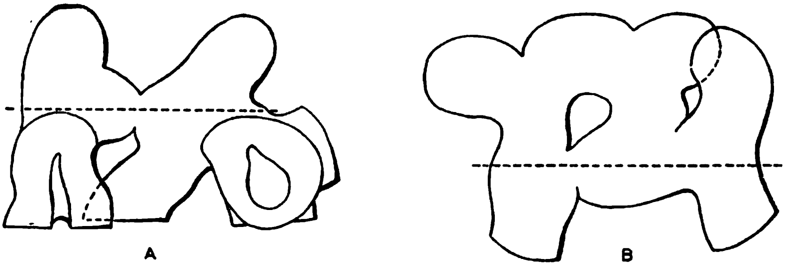

Fig. 3 Graphic reconstructions of anastomosing hepatic tubules in Acanthias. Detailed drawings of the sections indicated with dotted lines will be found in plate 2. *A*, tubules from an embryo 19 mm. long (S.C. 3) (see fig. 26). *B*, tubules from an embryo 19 mm. long (S.C. 2) (see fig. 27). × 100.

4. Later history of the hepatic cylinders

a. Growth of the hepatic network. Immediately after anastomosis has occurred the liver increases in size very rapidly. At first this growth is due almost entirely to the increase in number of the hepatic cylinders, but later the greater portion is brought about by the tremendous enlargement of the hepatic sinusoids. After anastomosis new cylinders are added to the existing network in three ways: by the formation of tubules from the remains of the hepatic pouches, by the formation of blind sprouts or buds from the sides of cylinders forming the network and by the production of new cylinders at the periphery of the network from the cylinders located there. These methods of addition to the hepatic network cease in the order given and the proportional amount contributed to the network by the several methods is in inverse order to that in which they are listed above.

The direct production of new hepatic cylinders from the hepatic pouches contributes but little to the bulk of the hepatic network and continues for only a short period after anastomoses are formed. As described in section 2, there are numerous tubules which arise at the bases of or between tubules which have previously sprung from the hepatic pouches. These younger tubules are at first small nipple shaped elevations arising singly and not from ridges. So long as the earlier tubules remain simple the later tubules follow them closely in their development, but when anastomoses become common among the older tubules and the sinusoidal circulation is well established, the younger tubules anastomose almost immediately after their formation either with one another or with cylinders of the already established network. In later stages hepatic tubules may sometimes arise as loops, the ends of which are attached to the wall of the hepatic pouch. By the time the embryo reaches a length of approximately 20 mm. (Normal plate Nos. 27–28, Balfour's stage N) the hepatic pouches are transformed into veritable hepatic ducts and thereafter no new hepatic tubules arise from them.

Hepatic cylinders, as has been seen, give rise to secondary buds while still in the form of single tubules, and this budding process continues long after anastomosis. The process can be most clearly demonstrated by the means of thick sections of which figure 41 from an embryo 24 mm. in length is an example.

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[4] The method of preparation of the thick sections used in this study was as follows: The embryo was infiltrated with celloidin and cemented to a piece of infiltrated spleen or liver which in turn was fastened to a fiber block. The specimen was then cleared by Gilson's method, which makes the block almost transparent. The block was then placed in the microtome clamp and a strong beam of light from a condenser directed upon the object. A Greenough binocular microscope equipped with low power lenses was set up over the object. With this arrangement it was possible to follow in detail the process of section cutting. Sections were then carefully cut away until the exact region desired was reached. By focusing with the binocular it was possible to determine the approximate thickness of the section needed to just include the desired structures, and this section was then removed with a single cut. Sections made in this way are often superior to reconstructions for the study of the form of very small structures. They are best stained in a very dilute carmin solution and cleared for a long period in cedar oil, after which they can be observed with the binocular microscope, or better with the aid of an Abby binocular eyepiece.

In the area of approximately 1 mm. represented here there are five blind buds projecting from the cylinders into the sinusoids. This method of addition to the network continues for a considerable period. I have found no traces of new buds in the body of the liver after the great increase in size of the cylinders when the embryo reaches a length of about 40 mm. However, in the portions of the liver which are the last to be formed, i.e., the dorsal margins and the posterior tips of the lateral lobes, this method of cylinder formation continues until the embryo reaches a length of 50 to 60 mm.

While in the earlier stages of the development of the hepatic network the increase comes perhaps equally from peripheral and interstitial growth, in later stages the latter method is by far the more important. The hepatic network terminates peripherally in a large number of blind knobs which by their growth and division give rise to a large amount of hepatic tissue. The cells of these terminal knobs remain in a comparatively undiffentiated condition while those of the more central part of the network are undergoing rapid changes in structure. Figure 43 shows a small portion of the tip of the lateral lobe of an embryo 20 mm. long. The mesothelium covering the liver has been removed. Here the terminal knobs are seen projecting from the general network and are often attached to the mesothelial sheath by strands of mesenchyma. This specimen was prepared by cutting a thick celloidin section of the region desired by the method already described. The celloidin was then dissolved away and the mesothelial covering stripped from the fragment with fine forceps.

The peripheral addition to the hepatic network takes place at first over the entire surface of the liver. But like the interstitial method of addition it is later limited to the tips of the lateral lobes and to the dorsal margins of both the body and lateral lobes of the liver which at a comparatively late stage grow rapidly upward between the stomach and the lateral body walls. In embryos 60 mm. in length these areas are much restricted and they cannot be seen in an embryo 80 mm. long.

In the rapidly growing parts of the liver the cylinders often terminate peripherally in expanded end-bulbs or vesicles which contain a lumen many times the diameter of that of the typical cylinder. Such vesicles are found in embryos from 25 to 40 mm. in length but not in older specimens. Their walls consist of a low columnar or cuboidal epithelium. Figure 4 is a cross section of one of these structures from the lateral lobe of an embryo 36.6 mm. long (S.C. 10). The significance of these vesicles is unknown to me.

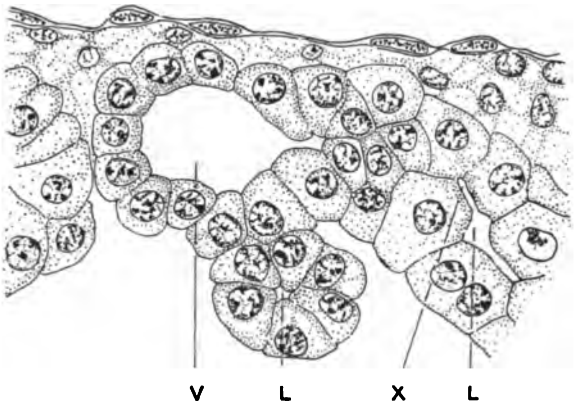

Fig. 4 Terminal vesicle of a hepatic cylinder from the lateral lobe of an Acanthias embryo 36.6 mm. long (S.C. 10). × 400. *L*, lumen of hepatic cylinder; *V*, terminal vesicle; *X*, side branch from central lumen of cylinder.

b. Changes in the size and structure of the hepatic cylinders. When the hepatic tubules enter upon anastomoses they are irregular in form and of variable caliber. The lumina of the tubules are large and irregular and are generally surrounded in cross section by 14 or 15 cells, if one may judge from the number of nuclei present, for cell boundaries are often indistinct at this time.

Table 1 shows some of the changes which take place in the course of the later development of the cylinders. The measurements and cell counts given in this table are in each case averages determined from twenty fair cross sections of cylinders of the posterior part of the median lobe of the liver. The cross sections of cylinders were taken at random from this region, except that those lying in the rapidly growing peripheral zone were avoided in each case.

In the first specimen of the series the number of cells bounding the lumen in cross section averages 12.5. The diameter of the tubules has become more uniform and averages 58 micra. Examples of such tubules are illustrated in figure 31. At about this time the liver begins to increase greatly in size. This growth is due in part to the actual increase in number of hepatic cells, as is indicated by the presence of numerous mitotic figures in the hepatic cylinders, but a much greater part of the increment is due to the establishment of the huge hepatic sinusoids. With this increase in size of the sinusoids the tubules are distinctly reduced in size, their average diameter dropping from 58 micra in an embryo 15 mm. in length to 37.6 micra in one 25 mm. and 35.6, in one 28 mm. in length. This reduction in diameter may be due somewhat to the decrease in the size of the lumen, but is caused mainly by the actual decrease in the number of cells surrounding the lumen at any one plane. The process is a continuous one after anastomosis is established, but is more rapid

TABLE 1

Measurements of the hepatic cylinders in Acanthias embryos

LENGTH OF EMBRYO IN MM.	AVERAGE NUMBER OF CELLS IN CROSS SECTION OF TUBULE	AVERAGE DIAMETER OF TUBULES IN MICRA
15.0	12.5	58.0
20.6	10.0	50.0
25.0	8.0	37.6
28.0	7.0	35.6
33.0	6.6	37.0
37.0	6.0	38.5
47.0	5.2	40.0
50.0	4.6	46.0
60.0	5.3	39.0
80.0	5.0	43.0
95.0	3.7	54.0

at first, dropping from an average of 12.5 in an embryo of 15 mm. to 7 in one of 28 mm. I think that this rapid and early reduction is due to the stretching of the tubules caused by the dilatation of the sinusoids among them. The tubules are attached to each other and to the mesothelial wall of the liver by strands of mesenchyma which may aid in the process by pulling upon the cylinders as the mesothelial wall is rapidly stretched in all directions. These mesenchymal strands can be seen in figure 43 of the liver of an embryo 20 mm. long.

After the embryo reaches a length of from 28 to 30 mm. the hepatic cylinders again begin to increase in size. They rise rapidly in diameter from an average of 35.6 micra in an embryo 28 mm. long to 46 micra in an embryo of 50 mm. and 54 micra in an embryo of 95 mm. This growth is still noticeable in an embryo of 220 mm. in which, however, the cells were too collapsed to admit of accurate measurement. While the diameter of the tubules increases, the size of the lumen and the number of cells surrounding it at any given plane as steadily decreases. Thus the average number of cells seen in cross section of a cylinder at 25 mm. is 8, at 50 mm. 4.6, and at 95 mm. 3.7. This indicates that the increase in size of the cylinder is due to the growth of the individual cells forming it and not to their multiplication, as is the early increase in size found in embryos from 13 to 16 mm. long.

Almost all this growth is due to the deposition of fat in the hepatic cells, the nuclei of which remain stationary or actually decrease in size. While the cells begin to increase in size even when the cylinders are becoming reduced in caliber, due to factors already mentioned, this growth is not sufficient to make up for the reduction until the embryo reaches a length of more than 30 mm.

The thick sections illustrated in figures 40, 41 and 42 show graphically some of the changes just described. Figure 40 is a section of a liver in which the process of anastomosis is well under way. It shows the large size and irregular caliber of both the cylinders and their lumina. Figure 41 is from a specimen in which the hepatic sinusoids have nearly reached their highest development and in which the cylinders are reduced to slender tubes. Figure 42 is from a somewhat older specimen in which the cylinders have again begun to increase in diameter. The changes in the number of cells surrounding the lumen of the cylinder in cross section are shown in the four figures forming plate 3.

My remarks upon the changes in the finer structure of the hepatic cells can be regarded as little more than notes made in the course of the general study of the growth of the cylinders.

During the development of the hepatic tubules and cylinders, the nuclei of the cells forming them are modified in shape, size and structure. At the time when the tubule ridges first appear upon the hepatic pouches the hepatic nuclei are elongately oval in outline, their longer axes averaging from 14 to 15 micra and their lesser axes from one-third to one-half of this length (fig. 28).[5] With the definite outpouching of the hepatic tubules the nuclei become broader and shorter, but their volume remains practically unchanged (figs. 29, 30). In an embryo 10 mm. in length (S.C. 20) from which tubules have been described and figured in the preceding part of this paper, the hepatic nuclei average 8 micra in diameter and 11 micra in length. Anastomosis has apparently no effect upon either the size or structure of the nuclei. In embryos from 10 to 20 mm. long one can follow the change in shape of the majority of the nuclei from broadly oval to spherical bodies. In a 20.6 mm. embryo (H.E.C. 1494, fig. 32) the great majority of the nuclei are spherical and have a diameter of 10 micra. Thereafter they gradually decrease in size even as the cells grow in size through an increase in fat content. In an embryo 47.3 mm. long (S.C. 11) the average diameter of the nuclei is 7.5 micra and in one of 95 mm. (H.E.C. 1882) it is little if any less. As the fat accumulates in the liver cells the nuclei may again change in shape, being in many cases pressed against the margin of the cell and assuming an oval or crescentic outline suggesting the form of the nuclei found in true fat cells. The nuclei which remain spherical have an average diameter of 7 micra or a little less (in embryos 200–240 mm. long).

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[5] All measurements given here were made from paraffin sections.

In early stages the nucleus is always in the basal portion of the cell. After anastomosis they are found more centrally located. In later stages they are found scattered, sometimes in the center of the cell surrounded with a film of protoplasm from which threads extend to the cell periphery; often close to the lumen wall of the cell and sometimes near some other point in its periphery.

Before the tubule anlagen appear the hepatic nuclei contain one, two or three large masses of chromatin which surround nucleoli and are generally applied closely to the nucleus wall. These chromatin masses are round or oval in shape and smooth in outline except for one or two small chromatin threads which may extend outward from each mass. The remaining space of the nucleus is filled with a clear nuclear sap through which run a few delicate and faintly staining fibrils. Such nuclei are characteristic of the young Acanthias embryo being found also in the cells of the mesenchyma, mesothelium, walls of the medullary canal and in the mesonephric tubules and duct. Those of the mesenchyma have been fully described by McGill ('10) in a study of the development of the striated muscle of the oesophagus in the dogfish. The figures in Neal's ('14) recent work illustrate the similar structure of the nuclei in the nervous system. Figure 28 shows the structure of a number of these nuclei stained with iron-hematoxylin. As the hepatic cells are differentiated the chromatin masses above mentioned become more irregular and there are given off from them a number of chromatin strands which eventually form a coarse network. The chromatin masses are reduced in size and may become detached from the nuclear wall. They also may be somewhat broken up and present a granular appearance. On the other hand, the chromatin threads which have originated from them may fuse forming secondary and generally smaller karyosomes which do not surround nucleoli. These changes are shown in figures 31 to 34, 38 and 39. The adult hepatic nucleus is rich in chromatin which is arranged in a coarse network containing several karyosomes some of which are probably the remains of the original ones and some of which are the result of secondary aggregation of chromatin granules from the chromatin threads.

Most of these karyosomes are applied to the nuclear wall. The hepatic cell retains the typical embryonic nuclear structure much longer than do the cells of the mesenchyma, mesothelium, nervous system or urogenital system. This typical embryonic structure is lost first in the cells forming the gall bladder and major hepatic ducts, next in the minor hepatic ducts which are formed from cylinders already well started upon a development towards typical hepatic parenchyma, and finally from the hepatic cells proper.

As has been remarked, fat droplets as indicated by vacuoles in the protoplasm of the hepatic cells appear, when the embryos obtain a length of about 25 mm. The use of special reagents would no doubt demonstrate the presence of fat prior to this stage. The droplets are found at first at the base of the cell, but later, in embryos 65 to 95 mm. long, droplets are found scattered through the entire cell body reducing the protoplasm to the network which has been described for the adult hepatic cell of selachians by Shore and Jones ('89) and Pilliet ('90).

In Acanthias embryos about 30 mm. long the gall bladder begins to press against the hepatic tissue which lies on either side and above it. This process is probably brought about by the great growth of the internal yolk-sac which lies below the gall bladder. This pressure of the gall bladder causes some degeneration of the hepatic tissue immediately surrounding it. Toldt and Zuckerkandl ('78) have described a similar process in the human embryo.

5. *Development of the hepatic tubules in other forms of selachians*

The development of the hepatic tubules in Torpedo, Raia and Mustelus differs somewhat from that of Acanthias. In these forms the lateral hepatic pouches do not reach the great development found in Acanthias and tubules are formed from these structures at a comparatively earlier stage. The omphalomesenteric veins are somewhat larger than in Acanthias and at the time when the individual hepatic tubules develop veinous channels are found both medial and lateral to the hepatic pouches.

The tubules which are formed from the dorsal parts of the pouches and of the pars hepatica medialis arise singly or in small clusters as do those in the same situation in Acanthias, but in the forms under discussion the dorsal or marginal tubules form a much greater part of the whole number produced than is the case in Acanthias. In Torpedo and in Mustelus the tubules from the ventral portion of the lateral pouches arise as in Acanthias from tubule ridges. These ridges are but slightly elevated and the corresponding grooves on the internal surfaces of the pouches are wide and shallow. The individual tubules which form from these ridges do not remain as mound-like structures arranged in rows but grow out almost at once and like the dorsal tubules appear as slender tubes extending outward between the veinous sprouts. In most cases the hepatic tubules which are first formed come in contact with the splanchnic mesothelium covering the liver.

The omphalo-mesenteric veins in these forms increase in size very rapidly and before tubule formation has progressed very far the anterior, ventral and lateral surfaces of the liver pouch are practically surrounded by a venous lake. All tubules which arise after this period project into this sinus and in their growth they push its endothelial wall before them. This process is illustrated in figure 44, which is of a thick frontal section of the liver of an embryo of Mustelus canis approximately 12 mm. long. The number of tubules arising directly from the pouch walls is small in these forms as compared with Acanthias for the surface area of the pouches is much reduced from the first. The large majority of the later tubules is formed by the branching of the earlier ones.

In Acanthias the hepatic tubules in the earlier period of their development are short, broad and pouch-like and the branches which arise from them are nipple-like projections. In the other forms studied both the primary and secondary tubules are slender elongated tubes which are early united in a complex branching and anastomosing network, the meshes of which are separated by sinusoids of large size. Figure 44 shows an anastomosis of a young hepatic tubule in Mustelus, and figure 45 of a similar section of an embryo about 4 mm. longer of the same species shows the complete establishment of the hepatic network.

While the early tubules of Raia, Mustelus and Torpedo are smaller in cross-section than are those of Acanthias, the lumina of these tubules are considerably larger. The cells lining the lumina are cubical or low columnar in outline as compared with the high columnar type found in Acanthias. The nuclear structure is quite similar to that of the hepatic cells in Acanthias, the chromatin covered nucleoli described for that form being particularly prominent in Torpedo and Mustelus.

The later history of the cylinders is quite similar to that of Acanthias. The cylinders rapidly increase in diameter and the contained lumina become smaller. The increase in diameter of the cylinders is due, as in Acanthias, to the growth of the individual cells, and the number of cells about the lumen in any given section steadily decreases. The nuclear changes are similar to those found in Acanthias. In such specimens of Mustelus and Squatina as I have examined the fat contained in hepatic cells remains in discrete droplets instead of forming one large mass as is generally the case in Acanthias. The same is true to some extent of Torpedo (fig. 35).

Pilliet ('90) has described areas of comparatively undifferentiated cells in the adult selachian liver. These cells form the portions of the cylinders which lie about the hepatic-portal veins. They are of comparatively small size and contain centrally placed nuclei which stain deeply with alum-carmin. The fat content of the cells is less than of those cells located elsewhere, and particularly of those lying in the neighborhood of the hepatic veins. Pilliet regards these smaller cells as reserves or nests of young cells which, from their position near the nourishing vessels, contribute to the growth of the organ. Apparently he did not find them in all the specimens which he examined.

I have seen no evidence of a retardment of the differentiation of the cells near the hepatic-portal veins in Acanthias embryos, and if such occurs it must be at a late period in the development of the organ. There are numerous small cells in the immediate neighborhood of the hepatic-portal vessels, but they all form portions of the terminal bile ducts, the development of which will be described in a later part of this paper. Such embryos of Torpedo as I have examined agree with Acanthias in lack of any definite nests or reserves of undifferentiated cells about the hepatic-portal veins. In well advanced Mustelus embryos, however, the hepatic cells which surround the larger branches of the hepatic-portal veins do remain somewhat smaller than those of the remainder of the liver and are not so completely charged with fat.

In summary it may be said that the chief differences between the two types of selachian liver, that represented by Acanthias and that by the several other forms mentioned, lies almost entirely in the earlier stages. These differences seem to be dependent on the difference in the size and arrangement of the omphalomesenteric veins at the time of the formation of the hepatic tubules.

DEVELOPMENT OF THE MINOR RAMI OF THE HEPATIC DUCTS

The formation of the minor rami of the hepatic ducts is closely associated with the history of the hepatic cylinders. In a preceding publication (Scammon '13) it was stated that all of the major and some of the minor rami of the hepatic ducts arise from the constricted bases of certain fairly definitely placed clusters of hepatic tubules. In following the history of these tubule clusters it was found that they become separated from the hepatic pouch from which they arise by a broad and rather indefinite peduncle which is at first hardly more than an extension of the pouch wall. Later this peduncle becomes constricted and elongated, forming a small branch from the pouch which by this time is transformed into a segment of the hepatic duct. This development begins before tubule anastomosis gets fairly under way and continues at the time when anastomoses are taking place. No new major rami arise as outpouchings after anastomosis is established and aside from the actual lengthening of already established rami, which is not great, all further growth of these structures takes place by the transformation of pre-existent hepatic tubules into ducts.

This illustrates well the two methods of hepatic duct formation. The first and more primitive type is that suggested by Minot ('93, p. 763) in which the duct is the result of a direct outpouching of the wall of the hepatic diverticulum. In the second and specialized type the duct is produced by the transformation of portions of the network of hepatic cylinders. Selachians clearly stand near the bottom of the scale in this phase of development. Here the ductus choledochus, the hepatic ducts and their major rami arise as outpouchings and only the minor rami and the most distal portions of the major ones are of trabecular origin. In ganoids, amphibians, reptiles and birds the ductus choledochus and the proximal part, at least, of the hepatic ducts are formed by outpouching and the remainder of the duct system from trabeculae. In the mammals apparently the ductus choledochus alone is the result of an outpouching, but further investigation may change this conception.

The differentiation of hepatic cylinders into bile ducts is very closely associated with their relations to the blood vessels. Bile ducts are only formed from cylinders which are in contact with the main trunks of the hepatic-portal veins or their larger and more definite branches. Still more striking is the fact that the side of the cylinder towards the vessels precedes in a very marked degree the differentiation of the opposite side and in fact in the smallest ducts the cells of the opposite side of the cylinder may never be transformed into duct epithelium at all but complete the ordinary development of true hepatic cells. Such a terminal duct from the liver of an embryo 95 mm. in length is shown in figure 37. On the other hand, of the many cylinders which closely surround the vascular trunk only a very small percentage is transformed into ducts. The development of the minor ducts is extremely small in proportion to the amount of hepatic parenchyma, smaller, I think, than in any other group of vertebrates. There is absolutely no indication of any system of intercalated ducts.

In the differentiation of a hepatic cylinder into a bile duct the former first approaches more nearly a perfect circle in cross section and the lumen distinctly enlarges. The cells on the vascular side of the lumen are reduced in actual height but become more columnar in form because of the still greater reduction of the size of their bases. At the same time the nuclei which in hepatic cylinders are round or broadly oval in section and lie near the center of the cells become elongately oval in outline and tend to retreat to the bases of the cells. The cells are so diminished in size that the nuclei which increase little, if any, in bulk almost fill them. The nuclei lose their typical structure of a clear karyoplasm containing one or two large chromatin masses from which radiate chromatin threads and present instead a reticular chromatin network made up of evenly distributed granules of about the same size. The protoplasm becomes homogeneous and colors darkly with plasma stains. An example of such a developing duct at an early stage is shown in figure 39. Approximately one-third of the duct which abuts upon a blood vessel shows considerable progress in differentiation, while the cells of the segment opposite it are true hepatic cells. Between the two are zones of transitional cells. To the side of this duct is a smaller one in still an earlier stage of differentiation. The outline sketch shown in figure 5 illustrates the changes in shape of the hepatic cells at the time of duct formation.

The cells of the large hepatic ducts which are formed from the hepatic pouches and their evaginations show much the same steps in cytomorphosis as do those just described. The nuclei of the larger ducts, however, are oval at the start and so undergo no changes in form, but the change in chromatin arrangement is the same as in the minor ducts. In the gall bladder the same changes also take place but at a late stage (60–80 mm.) the nuclei again become circular in cross section and come to occupy the centers of the cells which are much elongated.

It is sometimes stated that the bile duct epithelium is formed of cells of a more primitive type than those of the cylinders or trabeculae. In the forms under discussion, however, the bile duct cells have departed farther from the embryonic type than the parencyhmal cells, if we may judge by their nuclear structure.

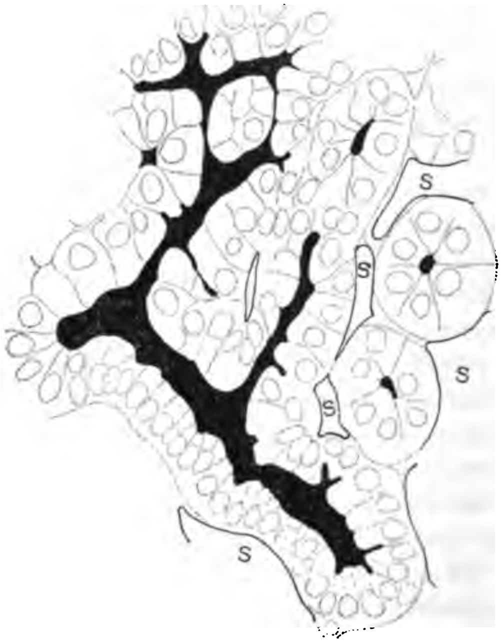


Fig. 5 Minor hepatic duct with connecting hepatic cylinders from the median lobe of the liver of an Acanthias embryo 37 mm. long (H.E.C. 353). × 400. Lumina of hepatic duct and cylinders solid black; hepatic cells in stippled outline; sinusoids, *S*, in unbroken outline.

DEVELOPMENT OF THE HEPATIC MESENCHYMA

It is well known that the mesenchymal tissue of the selachian liver is extremely scanty in the embryo although fairly abundant around the blood vessels in the adult. No particular study has been made of its origin, but Minot ('01) in his study of sinusoids, and more recently Debeyre ('09) in following the development of the hepatic cylinders, have noted the occasional delamination of mesenchymal cells from the mesothelium covering the liver and the presence of such elements along the walls of the hepatic sinuses. Debeyre also figures a cross section of a small portion of the periphery of the liver of an Acanthias embryo 30 mm. long, which shows small spurs of mesenchymal tissue extending inward from the mesothelium between the hepatic cylinders. The origin of the hepatic mesenchyma can be followed with little difficulty in Acanthias. Here it appears that this material is probably derived completely from the mesothelium but at two distinct periods and from two different regions, and that in both cases this proliferation is associated with distinct irregularities of splanchnic mesothelium.

The first mesenchymal proliferation appears at a little later time than the formation of the stroma of that portion of the gut which lies posterior to the liver. In Acanthias embryos 5 mm. in length the liver pouch is but slightly differentiated and consists of two shallow diverticula which lie in the lateral walls of the archenteron and are fused anteriorly to form the median hepatic pouch. The archenteron of this region is clothed on either side by a layer of splanchnic mesoderm which is continuous above with the radix mesenterica and below with the splanchnic layer of the blastoderm. At the radix this layer is much thickened but it is reduced to a moderately thin layer over the sides of the archenteron. The irregular endothelial walls of the omphalomesenteric veins intervene between the ventral part of the archenteron and the mesothelium, but no mesenchyma is present. Irregular processes from the mesothelium, however, do extend inward and in places it appears as though cells were about to be delaminated. A short time later the hepatic pouch increases much in size and extends anteriorly far in front of the anterior intestinal portal. With this change the ventral parts of the investing layers of splanchnic mesothelium are brought in contact and eventually fuse, thus forming for some time a ventral mesentery. In connection with this process there appear two distinct sets of mesothelial irregularities. These consist of the mesothelial villi on the right side and of numerous irregular folds on the left.

The mesothelial villi found in connection with the covering of the selachian liver were first described by Choronshitzky ('00), although they were observed in other forms long before that time. Hochstetter ('00) has given their later history in Acanthias in connection with his study of the formation of the septum transversum. As Hochstetter has stated, these structures are found only on the right side. They appear in embryos of 45 to 30 segments as a thickened plate of the mesothelium overlying the right omphalo-mesenteric vein (fig. 6 A). This plate is soon thrown into a series of pouch like irregularities, and the spaces thus formed are filled with a delicate network of protoplasmic processes from the mesothelial cells. The cores of the villi are thus at no time really empty, and are soon occupied by delaminated mesenchymal cells. From the bases of these villi mesenchymal cells are proliferated apparently both from the walls and the mesenchymal core, and soon come to form a small mass on the right side lying just below the omphalo-mesenteric vein. On the opposite side the irregularities are not villi but longitudinal folds. Unlike villi they do not arise from a thickened plate, the mesothelium at this point remaining as thin as elsewhere at the time of formation. Afterward the cells become more columnar and a mass of mesenchyma, larger but similar in other respects to that of the opposite side, is proliferated and underlies the left omphalo-mesenteric vein. Figure 7 shows the position and extent of these two mesenchymal proliferations in an embryo 7.5 mm. long (H.E.C. 1496). Soon after this stage the ventral mesentery breaks down and the ventral surface of the liver is free throughout its extent. With this process there is a delamination of mesenchymal cells along the median ventral line which unites the lateral ones already described, and there is thus formed a general ventral bed of mesenchyma which, as growth proceeds, forms a coating about the gall bladder and constitutes a large loose-meshed mass which extends forward from the gall bladder to the anterior mesothelial wall of the liver.

This constitutes the first and ventral contribution of mesenchyma to the liver from its mesothelial envelope. In its entire extent it produced the mesenchyma which surrounds the gall bladder, and later the vessels adjacent to it, the covering of the cystic duct, and to an undeterminable degree the sparse mesenchymal tissue of the lower part of the hepatic parenchyma. The irregularities of the mesothelium on the right side do not continue for any great period. By the time the embryo reaches

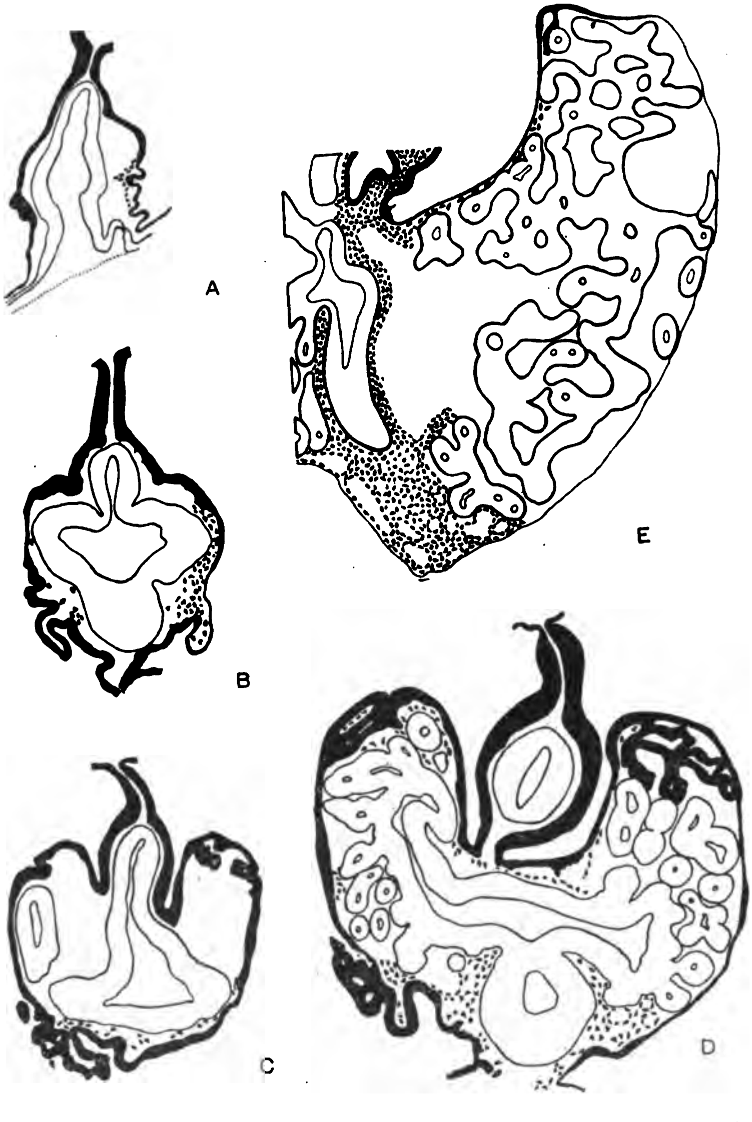


Fig. 6 A series of transverse sections of liver anlagen of Acanthias embryos of different ages. The mesothelium is represented in solid black, the mesenchyma in coarse stipple and the entodermal structures in outline; the blood-vessels are omitted. *A*, embryo 6.4 mm. long (S.C. 19); *B*, embryo 9 mm. long (H.E.C. 1495); *C*, embryo 10 mm. long (C.S. 20); *D*, embryo 15 mm. long (H.E.C. 1494); *E*, embryo 20.6 mm. long (H.E.C. 1494); all × 50.

a length of 15 mm. the mesothelium is smooth and much reduced in thickness. When the embryo reaches a length of 18 mm. the villi are reduced to small conical projections and are absent in embryos of 20 mm. Thereafter the mesothelium covering the ventral part of the liver on both sides becomes steadily reduced in thickness, and in embryos of 25 mm. and longer it is an exceedingly thin layer of squamous epithelium.

The second and dorsal mesenchymal proliferation begins at a later stage than does the ventral one. In an embryo 6.4 mm. in length (S.C. 19) (fig. 6 A) the dorsal portion of the mesothelium is seen to be slightly thinner than that of the ventral zone. As the lateral pouches of the liver are pushed upward, the omphalomesenteric veins come to lie mainly dorsal to these structures and the mesothelium over them is gradually thickened. This thickening is most noticeable at the lateral margins of the dorsal surface (fig 6 B). Soon after, in embryos of 10 to 12 mm., the mesothelium of this region becomes distinctly thickened in places and is invaginated forming tubules the walls of which are continuous with the splanchnic mesothelium and the lumina with the coelomic cavity (fig. 6 C and fig. 7). Often these tubules first appear as long trenches on the coelomic surface of the mesothelium. The sides of these trenches coalesce, thus roofing over the depression and forming tubules which open into the coelom at both ends. Other tubules grow in and end blindly. Examples of these early tubules are shown in figure 8. Although fairly regular at the start, these structures soon become irregular in caliber and form, and very often anastomose. Their lumina may be occluded in places thus forming mesothelial cysts which are generally connected by solid stalks with the covering mesothelium. At the time of their highest development the tubules form an anastomotic network which fills the dorsal fifth of the anterior part of the liver and they are then easily mistaken at first sight for true hepatic tubules (fig. 6 D). More careful study shows a number of differences between the two structures. Aside from the nuclear differences to be mentioned later, the mesothelial tubules are more irregular than the hepatic cylinders, their walls are thinner both actually and relatively as compared to their lumina, the cells are more columnar and there is no definite external cuticula as there seems to be in the hepatic cells at this time. The cytoplasm of the cells is also more granular. The mesothelial tubules may extend downward and come in contact with the hepatic cylinders, but generally they are replaced in this region by cords of mesenchymal cells. Figure 9 is a cross-section of a portion of the liver of an Acanthias embryo 15 mm. in length (H.E.C. 227) showing these tubules (*Mes.t.*) in the highest state of development.

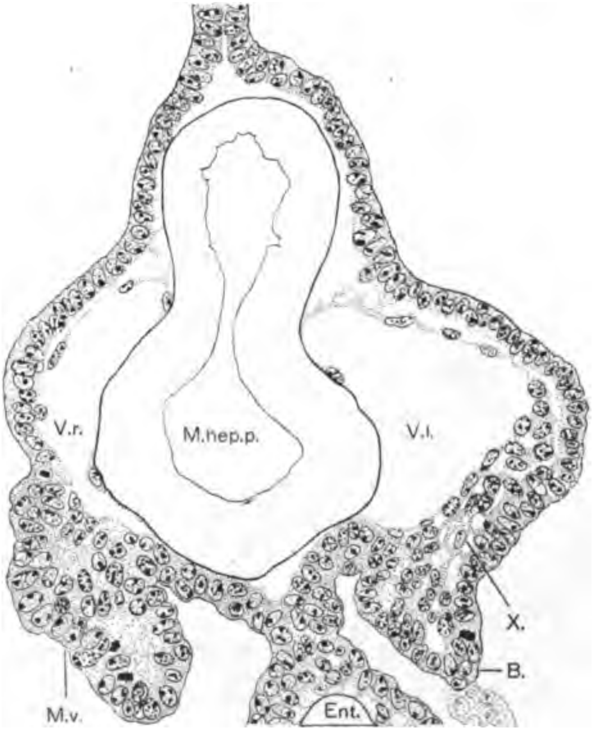
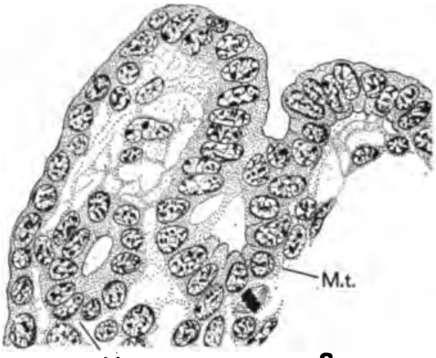


Fig. 7 Transverse section through the anterior part of the anlage of the liver of an Acanthias embryo 7.5 mm. long (H.E.C. 1496). × 400. *B*, tangential section of the mesothelial wall surrounding radicle of the left omphalo-mesenteric vein; *M.hep.p.*, median hepatic pouch; *M.v.*, mesothelial villus; *V.l.*, left omphalo mesenteric vein; *V.r.*, right omphalo-mesenteric vein; *X*, proliferated mesenchyma.



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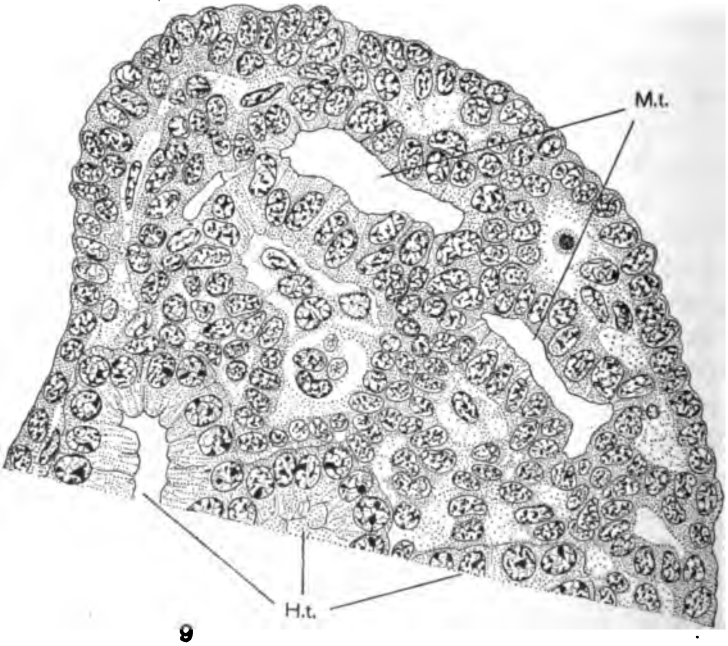

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Fig. 8 Transverse section of the mesothelial covering of the dorso-lateral margin of the liver of an Acanthias embryo 13.3 mm. long (S.C. 18). × 400. *M.t.*, mesothelial tubules.

Fig. 9 Transverse section of the dorsal portion of the liver of an Acanthias embryo 15 mm. long (H.E.C. 227). × 400. *H.t.*, hepatic tubules; *M.t.*, mesothelial tubules.

The mesothelial tubules do not remain long as such. The lumina are soon obliterated and the solid cords thus formed break up into mesenchymal strands. This process begins at the inner end of the cords and can be seen in figure 9. By the time the embryo reaches a length of 23 to 25 mm. the cords are entirely replaced by such mesenchymal strands which run in among the tubules which have now invaded this region (fig. 38). At 28 to 30 mm. even these strands have entirely disappeared and mesenchymal cells can be found only occasionally. The formation of mesenchyma by tubular ingrowths of mesothelium does not preclude the occasional delamination of single cells from the splanchnic mesoderm direct, but this delamination contributes but little to the total of the hepatic mesenchyma.

The nuclei of the splanchnic mesothelium in embryos 5 or 6 mm. in length, before mesenchymal delamination begins, are broadly oval in outline. They have the typical structure which is found in the nuclei of most of the tissues at this time. Each nucleus contains two or three large masses staining black with Heidenhain's hematoxylin. Each of these masses consists of a nucleolus surrounded by a thick layer of chromatin. The remainder of the nucleus is filled with a clear karyoplasm with a few faint strands of chromatin. These nuclei have been well described by McGill ('10) who studied the mesenchyma of the foregut region in the dogfish. As cells are delaminated from the mesothelium and as the mesothelial tubules are broken up into a mesenchymal syncytium, most of the nucleoli disappear and the chromatin is gradually scattered in a finely divided network. Similar changes take place in the nuclei of mesothelial cells but at a later period. A comparison of figures 7 and 38 will show the two extremes of this change. Mitotic figures are numerous in the mesothelium but scarce in the mesenchyma derived from it.

The relation of the mesothelial tubules to the sinusoids is of interest in the light of Bremer's ('14) recent work on the earliest blood vessels in man. Bremer found mesothelial cords from the extra-embryonic coelome connected with the angiocysts and solid cords of the vascular net in the body stalk, and he suggests that the elements of the vascular net arise from these ingrowths.

I have not found that the cavities in the mesothelial tubules or cysts of the selachian liver connect with the blood spaces. As the walls of these structures break up into mesenchymal strands it is impossible with ordinary section and staining methods to distinguish between them and the endothelium surrounding the adjoining sinusoids. This is particularly true if one tries to follow the mesenchymal strands which penetrate between the hepatic cylinders in later stages such as the one shown in figure 38. The mesenchymal and endothelial cells form free anastomoses. McGill ('10) apparently found the same condition in a much younger embryo, judging from figure 9 of her paper with which the section shown in my figure 7 is in agreement.

The reduction in the thickness of the mesothelial covering of the liver and the disappearance of the mesothelial villi is no doubt due in part to the great growth of the contents of the former, particularly of the hepatic cylinders and sinusoids. This increase takes place rather suddenly in embryos from 18 to 20 mm. in length and the reduction in the thickness of the mesothelium is to some extent coincident with it. It is noticeable, however, that in many cases when a hepatic cylinder comes in contact with the covering mesothelium the latter layer is distinctly reduced in thickness, its constituent cells change from a high columnar to a cuboidal form and the nuclei from oval to nearly spherical bodies. This change is probably not due to pressure for in places where two tubules come in contact with the mesothelium and are separated by a distance hardly equal to their own diameter the mesothelium becomes thin at the points of contact and remains thickened over the small intervening portion. Were the effect of tubule contact only a matter of pressure, one might expect some flattening of this intervening place as well. Also the mesothelium is not pushed outward at these points of contact but remains on a level with the surrounding surface.

One is tempted to suggest the homology of this dorsal mesenchyma-producing mass with the 'Vorleber' of Kölliker and His. But this similarity is only a superficial one as the mass just described does not appear until after the liver is well developed, while in birds and mammals the 'Vorleber' is a precursor of that organ. Also the selachian mesenchymal mass lies in the dorsal and posterior part of the liver along its free upper surface, while the 'Vorleber' lies anteriorly and is broadly connected with the dorsal wall of the coelom. Ziegler ('88) showed long ago that the mesenchyma of the intestine in selachians arose from two longitudinal zones of proliferation from the splanchnic mesoderm. One of these zones lies above the archenteron and the other ventral to it. The method of development of the hepatic mesenchyma indicates that these dorsal and ventral zones of proliferation extend forward into the liver region as well and remain distinct for a considerable period even after the liver has separated from the gut above it. A comparison of a semi-diagrammatic figure of a cross-section through the hepatic region with Ziegler's diagram of a similar section through the middle of a trunk segment shows the essential agreement in the origin of the splanchnic mesenchyma in the two regions (fig. 10).

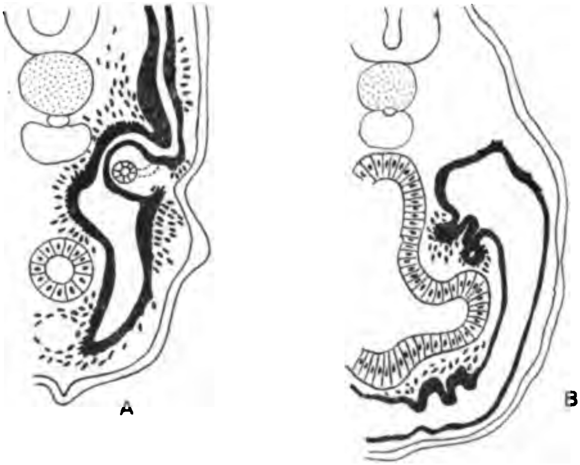

Fig. 10 A comparison of Ziegler's figure: *A*, of the mesenchymal tissue in the trunk region of a selachian embryo with a semi-diagrammatic figure; *B*, showing the origin of the mesenchyma in the hepatic region of an Acanthias embryo.

HISTORY OF THE HEPATIC SINUSOIDS

The sinusoids of the selachian liver are most striking objects and at one time form decidedly more than half of the entire bulk of the organ. Minot ('00) has given the only description of them which is at all complete as a part of his exposition of the nature of the sinusoidal circulation. Brief notes are also to be found in the papers of Holm ('97) and Debeyre ('09). The general development of the veins of the liver in these fishes has been studied by Rabl ('92), Hoffmann ('93) and Hochstetter ('93). The early development of the omphalo-mesenteric veins has been observed in detail by Mayer ('87) and by Ruckert ('88). Therefore I shall give only the briefest outline of the general history of the veins, and will begin with the conditions found in Acanthias embryos from 8 to 10 mm. in length. In such embryos there are paired omphalo-mesenteric veins extending forward to the sinus venosus on either side of the gut. The left vein is somewhat larger than the right. At first the right and left trunks are quite separate but soon they form two anastomoses, one posterior to the pancreas and the other, a little later, just posterior to the liver below the foregut. From the latter anastomosis the veins pass forward on either side of the foregut and medial to the lateral hepatic pouches until they reach the anterior end of the liver where they join and form the sinus venosus. It is important to note that in Acanthias in early stages only the medial surfaces of the lateral pouches are in contact with vascular channels. There are no vascular channels between the lateral surfaces of the hepatic pouches and the mesothelium covering them.

Figure 11 A shows a reconstruction of a somewhat later stage in which important changes have taken place. Now only the left omphalo-mesenteric vein passes through the groove between foregut and lateral hepatic pouch to join the sinus venosus. The right vessel ends blindly anteriorly and is connected with the left by the large anastomosis which lies behind the liver. In the meantime either a single vessel or several vascular sprigs have grown back on either side from the sinus venosus and passing

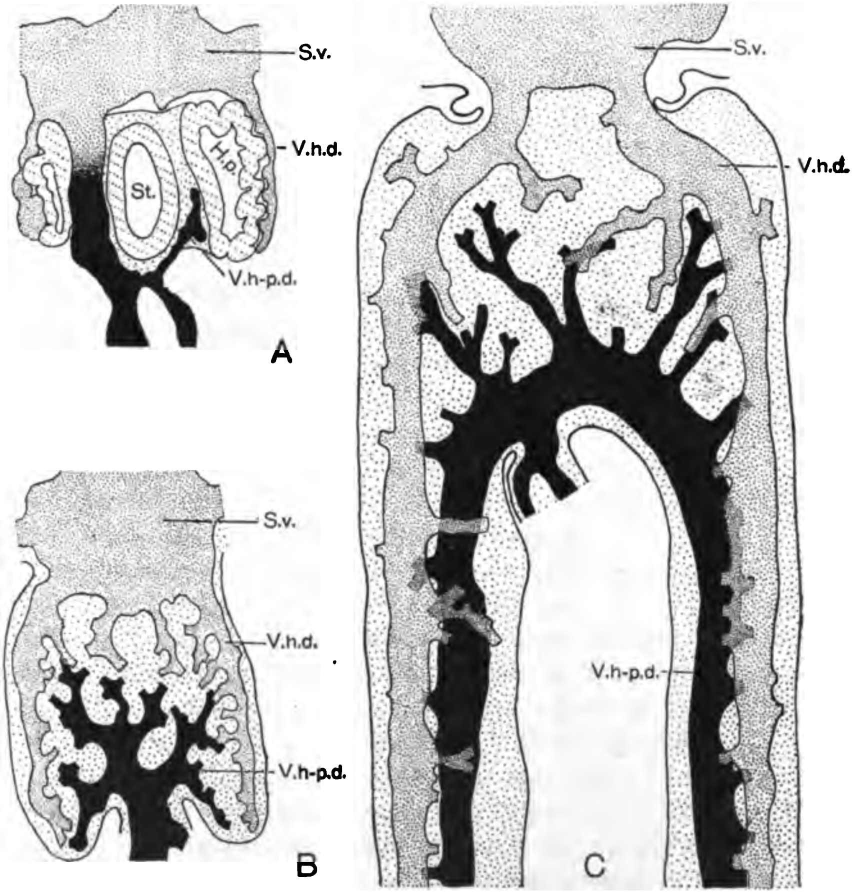

Fig. 11 Semi-diagrammatic graphic reconstructions (from frontal sections) of the liver veins, as seen from above, of three Acanthias embryos. The hepatic-portal veins are represented in solid black, the hepatic veins and sinus venosus in heavy stipple and the hepatic parenchyma in light stipple. In figure 12 A, the connection between the liver and the foregut and the upper parts of the lateral hepatic pouches are represented as cut away dorsally. *A*, embryo 15 mm. long (H.E.C. 230); *B*, embryo 20 mm. long (S.C. 31); *C*, embryo 41 mm. long (H.E.C. 371). *H.p.*, right lateral hepatic pouch; *St.*, stalk connecting liver pouch with foregut above; *S.v.*, sinus venosus; *V.h.d.*, right hepatic vein; *V.h.-p.d.*, right hepatic-portal vein.

along on the lateral surfaces of lateral hepatic pouches have formed a vascular plexus consisting of several large sinuses interrupted by small lacunae. In the drawing the omphalo-mesenteric veins, properly speaking, are represented in solid black and the sinus venosus and the veins developed from it in stipple. The posterior part of the omphalo-mesenteric veins may now be called 'hepatic-portal' veins, this being the term commonly used to designate them in the adult, while the veins which are efferent in their drainage will be designated by the term hepatic veins, which is the name applied to them in works on adult anatomy.

In the stage just described tubules have formed only on the lateral surfaces of the hepatic pouches. Soon thereafter tubule formation takes place with great rapidity along the margins of the pouches and from the pars hepatic·medialis as well. The latter process results in breaking up the clear passageway of the blood along the left omphalo-mesenteric vein to the sinus venosus and in place of this one discrete passage there are formed a number of small irregular venous channels which pass from the posterior portions of the omphalo-mesenteric veins (hepatic-portal veins) in among the tubules and join the hepatic veins.

At this time there is a marked growth of the posterior lobes of the liver which brings about several changes in the position of the venous trunks. These may be seen by comparing figures 11 B and 11 C which are graphic reconstructions of embryos 18 mm. and 41 mm. long respectively. The branches of the early hepatic-portal vein which extend backward along the inner surface of the posterior lobes assume more and more importance, becoming in the later stage the posterior continuations of the main trunks of the vein. The former main trunks which extended forward are relatively reduced in size and appear as branches of these posterior vessels. At the same time the sinus venosus becomes more completely separated from the liver as the septum transversum is formed and the lateral hepatic efferent trunks converge towards the middle line. In so doing they take up the two larger venous radicles which formerly lay medial to them and represented the remains of the extreme anterior ends of the former omphalo-mesenteric veins. Thus these vessels, as may be seen by comparing *B* and *C* of figure 11, become branches of the hepatic veins instead of direct tributaries of the sinus venosus. In older embryos an increase in the caliber of the hepatic veins posterior to their entrance into the sinus venosus indicates the position of the hepatic sinuses which become so prominent in some selachians and which have been discussed in some detail by Neuville ('01).

In selachians as compared with the higher groups of vertebrates the primitive liver veins are retained in the adult almost in their entirety. The main vessels are established early before the sinusoidal complex is well developed and these trunks remain almost complete, although they may vary in size with changes in the hepatic parenchyma. In figure 14, for example, the main trunks of the hepatic vein can be seen in each section although the size of these trunks varies greatly. The only primitive hepatic vessel which is really completely broken up into sinusoids by the hepatic cylinders is the small segment of the left omphalomesenteric vein which passes over the pars hepatica medialis to join the sinus venosus and even the anterior and posterior ends of this vessel remain as branches of the left hepatic and the hepatic-portal veins respectively. The growth in length of the hepatic and hepatic-portal veins takes place at the ends of the lateral lobes of the liver. Here there exist venous sinuses which are joined by both veins and which are interrupted by only occasional hepatic cylinders sheathed with endothelium. From these sinuses the posterior ends of the afferent (hepatic-portal) and efferent (hepatic) veins are differentiated by the growth of a septum of hepatic cylinders which at first form a loose meshwork and later become a compact wall through which only small capillary sinusoids pass from one vein to another. Some of the venous branches of the second order represent remains of original venous trunks as for example the two largest tributaries of the hepatic veins from the median lobe. The others are the representatives of the larger passageways which have pushed between the fairly constant tubule clusters which arise from the hepatic pouches. Each of the larger branches of the hepatic-portal vein in the median lobe of the liver accompanies for a short distance a secondary hepatic duct derived from one of these tubule clusters. As a rule the tubule ridges of the lateral surfaces of the hepatic pouches are completely formed and are beginning to break up into rows of individual anlagen before these blood vessels invade their vicinity. Different embryos, however, show some variation in this respect. The vessels pass backward between these rows of tubules and form a vascular plexus interrupted by a few small lacunae. After this process is quite well under way, the main vascular trunk of the left omphalo-mesenteric vein is interrupted by the growth of the pars hepatica medialis and as a result vascular sprouts extend out from the posterior part of this vessel and its fellow of the opposite side. These sprouts join with the plexuses of the hepatic veins and there is thus produced a set of sinusoidal vessels connecting the remains of the omphalo-mesenteric (hepatic-portal) venous system with the true hepatic veins. The extension of this network is gradual, and is not completely established until the tubules are well differentiated and anastomosis between the tubules begins to take place. The establishment of the complete sinuosidal circulation and of tubule anastomosis is practically simultaneous. In Acanthias, as in the pig according to Hilton ('03) and in reptiles (Hammer '93), there is no reason to believe that the blood vessels have anything to do with the formation of the earliest hepatic tubules. Certainly there is no breaking up of a solid mass of liver cells as described in the frog by Shore ('93) or any indentation of the wall of the lateral liver pouches as has been seen in Torpedo by Choronshitzky ('00). The vessels last of all penetrate among the tubules of the ventral surface of lateral hepatic pouches. Figure 12 is a frontal section through the tubules of this region of an embryo 15 mm. in length (H.E.C. 228). It will be seen that the tubules are separated only by scattered mesenchymal cells with the exception of a single vascular sprig.

Minot ('00) has remarked that in the liver, and in the pronephros and mesenephros as well, the sinusoids which at first may be small, increase in size until they reach a certain maximum and then decrease. To secure some idea of the extent of the changes which take place in this process, the area in cross-section of the blood spaces of selected sections have been measured from a series of livers of selachian embryos. Eight sections were selected for measurement from each liver. To secure sections representing about the same relative planes in each specimen the following method was employed. The liver was considered as divisible into three parts, as represented in figure 13. The first segment (*A* to *B*, fig. 13) consisted of that part of the liver anterior to the foremost part of the curved cystic duct. The second segment (*B* to *C*) extended from the cystic duct to the

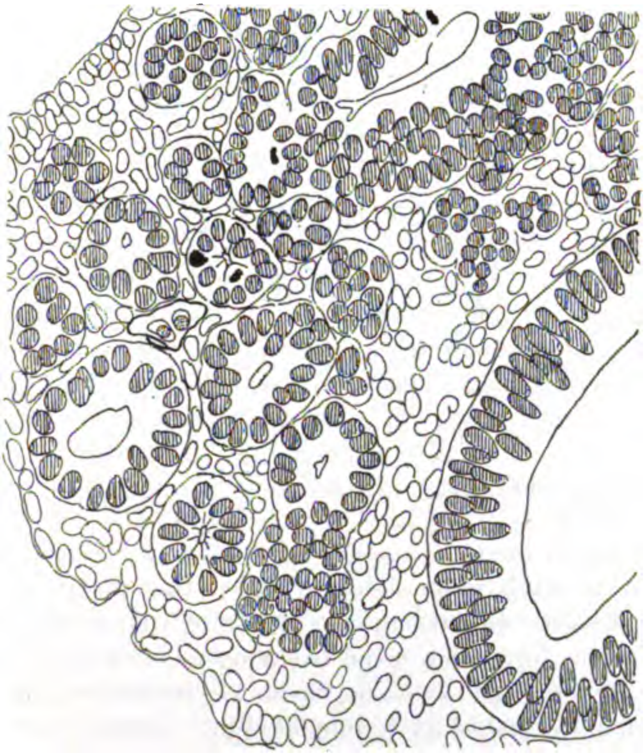

Fig. 12 Frontal section through the tubules arising from the ventral surface of the pars hepatica medialis of an Acanthias embryo 15 mm. long (H.E.C. 230). × 150. Hepatic cell nuclei are shaded with vertical lines, nuclei of mesenchymal cells drawn in outline. A single vascular twig is seen in cross section above the largest hepatic tubule.

plane where the anterior lobe of the liver joins with the two lateral and posterior ones. The two lateral lobes form the third segment (*C* to *D*). The first segment, *A* to *B*, was divided into three equal parts and the sections falling upon the planes separating these parts (*1* and *2*) were used for measurement. Two sections were selected in the same manner from the second segment *B* to *C*. The third segment *C* to *D* was divided into five equal parts and the four sections falling on the dividing planes used for measurement. In this segment only the left lobe was measured in each case. The sections thus determined were drawn with the camera lucida or projection apparatus and the total area, the total area of the vascular bed in cross-section, and the area of the larger vascular trunks in cross-section were then determined by means of a planometer.[6] From this data the figures given in table 2 were calculated. Such a method can lay no claim to great accuracy. The possibility of error is quite large and the method of determining the position of the sections has some objections, for all parts of the liver do not grow at the

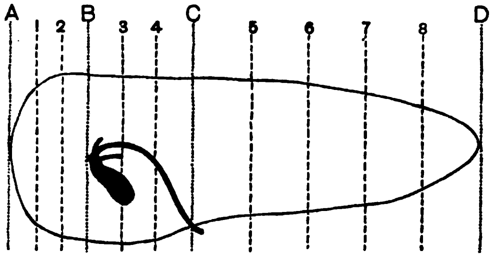

Fig. 13 Diagram of the liver of an Acanthias embryo showing method of selecting sections for measurements of the blood vessels. Bile ducts and gall bladder represented in black.

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[6] It is impossible to determine the exact area of the large trunks in the earlier stages because of their great irregularity and their many connections with the adjoining sinusoids. In embryos 20 to 21 mm. long the area of the main venous trunks forms about 20 per cent of the entire cross section area of the vascular spaces of the liver. This drops to about 15 per cent in embryos of 25 to 28 mm. and increases thereafter with the relative reduction of the sinusoids. In the last member of the series in table 2, the main trunks formed about 30 per cent of the total cross section area of the blood vessels.

same rate and the development of the vascular supply of the organ is closely related to its growth. Such a study, however, does give us some rough approximations which may be of value.

A study of table 2 brings out clearly the three stages in the vascularization of the liver in Acanthias. The first stage found in an embryo between 12 and 20 mm. in length is one in which the tubules are growing with great rapidity and the sinusoids are only cleft like endothelium lined spaces (figs. 14 A and B). It is only in the lateral lobes of the liver where the hepatic and the hepatic-portal veins unite in large sinuses that the vascular percentage of the organ is high. During the latter part of this period tubule anastomoses are forming in large number. With anastomosis the vascular supply of the organ increases with the greatest rapidity and the tubules, taking on the form of slender cylinders, are separated by very large vascular spaces. This phase, which is inaugurated rather suddenly, continues while the embryo grows from a length of about 20 mm. to a length of

TABLE 2

Measurements of the hepatic vascular spaces in Acanthias embryos

LENGTH OF EMBRYO IN MM.	DESIGNATION	AREAS IN MM.	LEVELS OF SECTION							
			1	2	3	4	5	6	7	8
15.0	H.E.C. 227	T.a.[1]	0.238	0.321	0.314	0.314		0.029		0.019+
		V.a.[1]	0.036	0.044	0.020	0.021		0.005		0.006−
		V.%[1]	**15.3**	**13.8**	**6.3**	**6.7**		**26.3**		**30.0**
20.5	S.C. 5	T.a.	0.308	0.423	0.581	0.502	0.160	0.099	0.065	0.027
		V.a.	0.030	0.047	0.031	0.112	0.172	0.024	0.012	0.007
		V.%	**9.8**	**11.2**	**14.0**	**22.4**	**10.8**	**24.5**	**19.7**	**27.7**
20.6	H.E.C. 1494	T.a.	0.738	0.942	0.742	0.934	0.412	0.232	0.234	0.178
		V.a.	0.349	0.373	0.385	0.392	0.172	0.124	0.141	0.112
		V.%	**47.3**	**39.7**	**52.0**	**42.0**	**41.8**	**53.4**	**60.3**	**63.5**
28.0	S.C. 6	T.a.	1.120	1.440	1.210	1.190	0.357	0.324	0.303	0.204
		V.a.	0.698	0.616	0.591	0.522	0.205	0.167	0.173	0.157
		V.%	**62.4**	**42.8**	**48.9**	**43.9**	**57.5**	**51.5**	**57.0**	**77.0**
36.6	S.C. 29	T.a.	2.060	2.120	2.340	2.270	0.669	0.595	0.590	0.357
		V.a.	0.403	0.578	0.559	0.644	0.152	0.108	0.084	0.051
		V.%	**19.6**	**27.3**	**23.9**	**28.4**	**22.8**	**18.3**	**14.4**	**14.4**

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[1] T.a., total area of cross section in square mm.; V.a., area of vascular spaces in square mm.; V.%, percentage of total area occupied by vascular spaces.

about 30 mm. During this period roughly one-half of the bulk of the liver is made up of vascular spaces and of this half much the greater part is in the form of sinusoids. Figure 14 C is a section through a plane corresponding to figure 14 B, and shows graphically the extent of the blood vessels at the time when they form the larger part of the liver. New tubules develop in great numbers during this time. Finally a period of reduction in the size of the sinusoids sets in. This reduction is brought about entirely by the increase in the size of tubules already formed. By this increase in the parenchyma the sinusoids are reduced to the 'capillary sinusoids' of Minot. This process is very noticeable in embryos between 35 and 45 mm. in length. It continues probably to the time of birth. Figure 14 E is of a cross section of the liver of an embryo 47.3 mm. long and shows the marked decrease in the size of the sinusoids at that time.

The increase in size of the sinusoids is both actual and relative. The decrease in the total area (in cross-section) of the sinusoids is at first only a relative one, but later, for a short period at least, it is actual as well as relative.

A point which table 2 does not bring out is that the size of the sinusoids is not determined by their position in relation to the larger vessels but is dependent upon the stage of development of the parenchyma with which they are interwoven. The sinusoids do not form a tapering system of vessels largest near the veins which receive them. This has been in a way pointed out in the section upon the growth of the hepatic cylinder network. In the lateral lobes for example, the growth of the tissue is at first entirely backward and during this period sinusoids of large size are generally distributed throughout the lobe (fig. 15 A). After the lobes have completed the greater part of their posterior growth there begins a great increase in the hepatic tissue along their dorsal margins and the lobes gradually push upward on either side of the intestine. With this change in the area of rapid growth there also occurs a change in the sinusoids which, as is shown in figure 15 B, continue to form practically half of the dorsal portion of the lobes while in the ventral portions they form less than 10 per cent of the total area in cross-section.

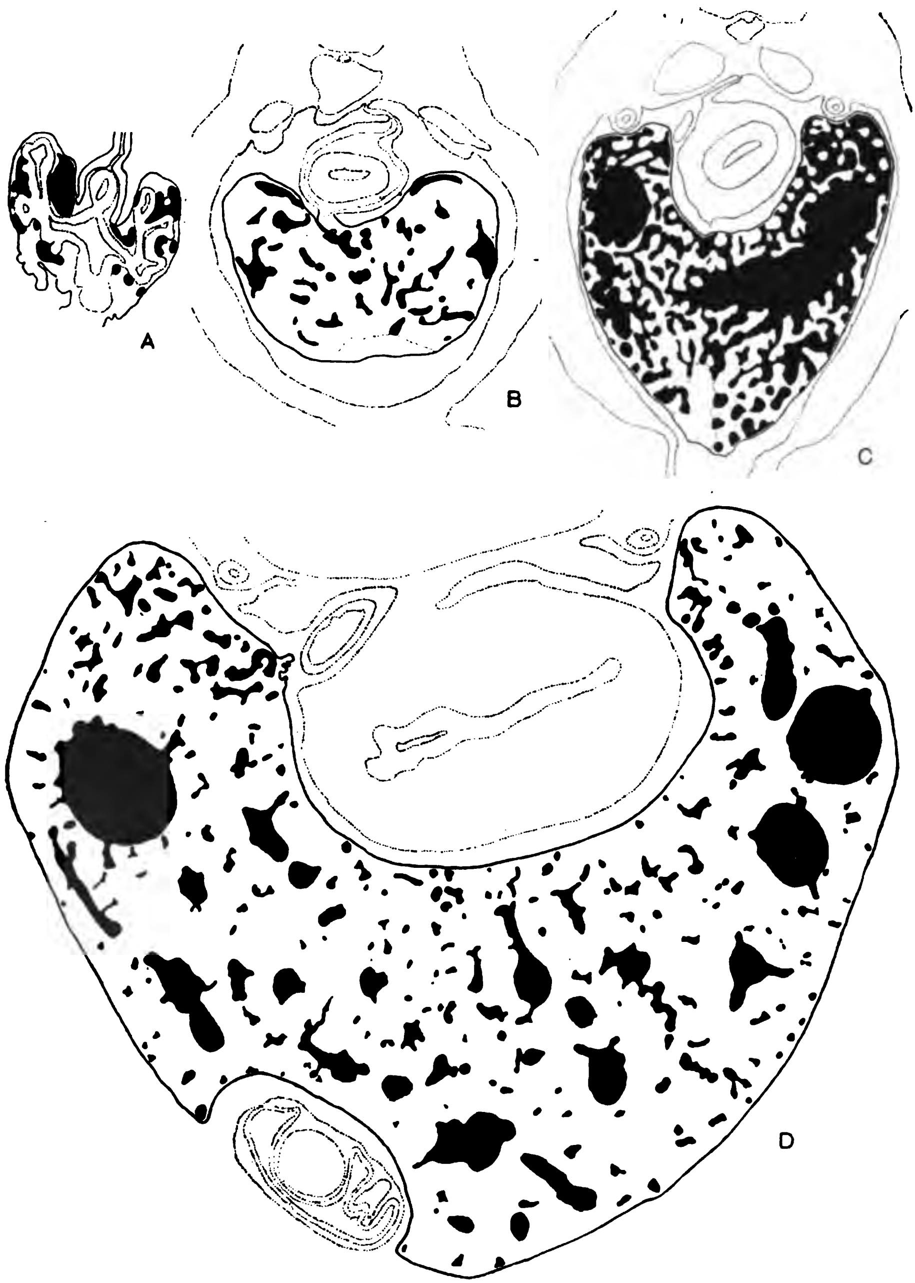
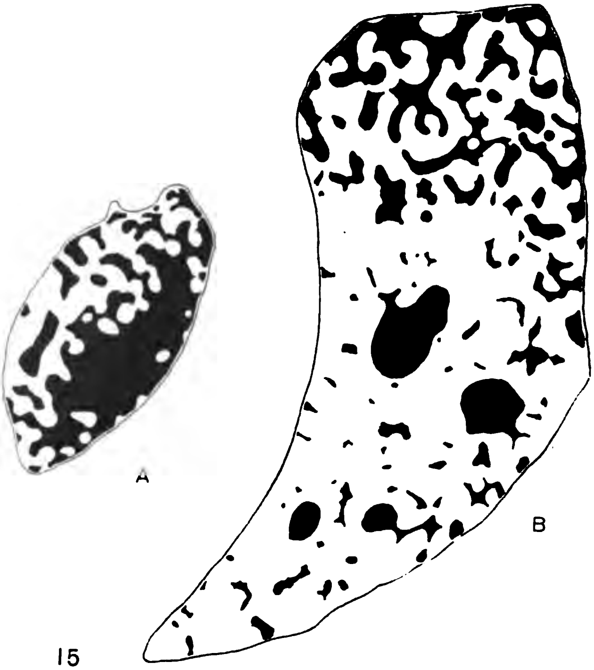


Fig. 14 A series of transverse sections of the livers of Acanthias embryos of different ages showing the development of the sinusoids which are represented in solid black. All sections, with the exception of *A*, are taken at a plane equally distant from the anterior end of the liver and the anterior end of the gall bladder; all × 35. *A*, embryo 13.3 mm. long (S.C. 18); *B*, embryo 20.5 mm. long (S.C. 5); *C*, embryo 28 mm. long (S.C. 6); *D*, embryo 47.3 mm. long (S.C. 11).

Similar changes are seen in earlier stages in the main median lobe of the liver and in the process of the cystic lobe which extends into the yolk stalk coelome.

In Mustelus and Torpedo the early development of the hepatic sinusoids is quite different from that seen in Acanthias. In these forms the omphalo-mesenteric veins become much enlarged at an early stage and lie both dorso-medial and ventro-lateral to the hepatic pouches instead of only medial to them in Acanthias. As a result the hepatic tubules are in contact with the walls of large vascular chambers from the time of their first appearance and as they grow they can only do so by pushing the endothelial walls of the vessels before them. The tubules, as has been remarked, are slender and elongated and unite by anastomoses soon after their formation. Thus there is no stage in these forms corresponding to the early one in Acanthias where the sinusoids are small cleft-like spaces. The proportion of the volume of the liver formed by sinusoids is very high at first but is steadily reduced as development proceeds. Figure 16 of two transverse sections of Mustelus embryos 16.7 mm. and 22.5 mm. in length respectively illustrates these points, as do also the thick frontal sections shown in figures 44 and 45. The larger vasculartrunks of the liver in Mustelus and Torpedo are mapped out by the growth of the hepatic cylinder network between them. As in Acanthias the main liver veins are marked out early in the development of the organ, and are not formed by the fusion of smaller vascular spaces.

In both the forms under discussion and in Acanthias the hepatic sinusoids are formed by the intercresence of the hepatic cylinders and the omphalo-mesenteric veins. In Mustelus and Torpedo this intercresence is due to the invasion of the space occupied by the vein. In Acanthias, *in early stages,* the intercresence is due to the penetration of the venous sprouts about the tubules which are already established. In later stages in Acanthias the cylinders increase by growing into venous spaces and the end result in either case is the same. Thus both the methods of sinusoid development postulated by Minot ('00) and by Lewis ('04) are found in these forms.



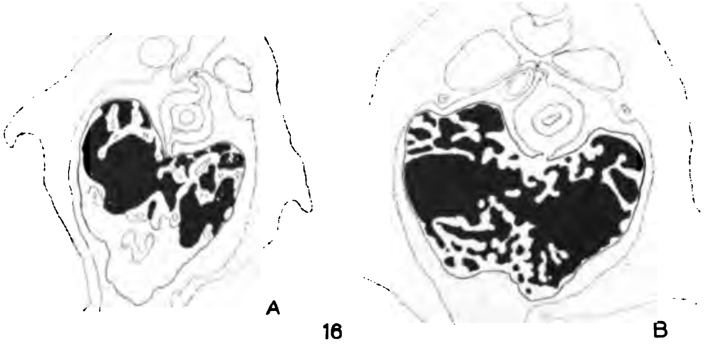


Fig. 15 Two transverse sections through the middle of the left lateral lobe of Acanthias embryos; both × 75. *A*, embryo 28 mm. long (S.C. 6); *B*, embryo 47.3 mm. long (S.C. 11); sinusoids represented in black.

Fig. 16 Transverse sections through the middle of the median lobe of two embryos of Mustelus canis. *A*, embryo 16.7 mm. long (S.C. 32); *B*, embryo 22.5 mm. long (S.C. 33); both × 35; sinusoids represented in black.

In Acanthias the establishment of the sinusoidal circulation and the process of anastomosis of the hepatic tubules occur almost simultaneously. In Mustelus and Torpedo the hepatic tubules anastomose immediately after their intercresence with the omphalo-mesenteric veins. At first sight there would seem to be some relation between the sinusoidal type of circulation and the anastomosis of glandular end-pieces. This seems to be supported by the evidence that the pancreas of ganoids has free anastomoses with a sinusoidal circulation and these conditions are also found in the paraphysis of Necturus (Warren '05) and in the islands of Langerhans in Mammalia (deWitt '06). Sinusoids also occur in other ductless glands which anastomose. On the other hand I have found no report of anastomoses between the tubules of the mesonephros, and a free sinousoidal circulation exists in this organ. The tubules of the embryonic mammalian pancreas are known to anastomose freely, and there is no record of a sinusoidal circulation in this organ. Similarly Braus ('00) finds a free anastomosis of the end pieces of the bulbo-urethral gland which has the capillary type of circulation and Bremer ('11) has found the same conditions in the testis.[7] It is evident that no general rule can be laid down regarding this relation, at least with our present knowledge of the subject.

SUMMARY

A. Development of the hepatic parenchyma

1. Hepatic tubules are first represented by longitudinal ridges formed on the external surfaces of the pars hepatica lateralis and medialis, and by slight irregularities of the margins of the pouches forming the pars hepatica lateralis.
2. The hepatic ridges are converted into irregular rows of tubule anlagen by transverse constructions.
3. The individual tubule anlagen thus formed grow outward and are differentiated into expanded terminal chambers and

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[7] Occasional anastomoses are found in a number of glands such as the gastric and uterine glands. It is to be expected that such occasional connections will be found in almost any gland which is studied carefully with the aid of reconstructions.

constricted proximal necks. Secondary tubules of the second, third or fourth order may be produced from the terminal expanded chamber before tubule anastomoses are formed.

4. In Acanthias the hepatic tubules may differentiate in regions into which blood bessels have not penetrated.

5. Tubule anastomosis and the complete establishment of sinusoidal circulation are practically simultaneous.

6. Anastomoses of tubules may take place end to end, side to end, or side to side. The frequency of the several types is in the order stated.

7. The process of anastomosis involves the stages of (a) the contact and fusion of the tubules involved, (b) the rearrangement of cells in the area of confluence, and (c) the establishment of a new connecting lumen.

8. While the cell position is shifted in anastomosis, the cell axis remains unchanged.

9. After anastomosis the increase in the number of hepatic cylinders takes place in three ways, viz: (a) the formation of hepatic cylinders directly from the pouch wall; (b) the interstitial development of cylinders from blind sprouts from the network; (c) the peripheral growth of the terminal hepatic cylinders.

10. These methods of increase cease in the order named and contribute to the network inversely to the order given above.

11. Increase in the number of cylinders of the hepatic network first ceases in the body of the median lobe and last in the tips of the lateral lobes and along the dorsal margins of the lateral lobes and connecting portion.

12. The number of cells surrounding the lumen of the hepatic cylinder in cross-section drops from an average of 12 + in the tubule at the time of anastomosis (14 to 15 mm.) to 3 ± in an embryo 95 mm. long.

13. The diameter of the hepatic cylinders increases up to and immediately after the time of anastomosis. With the enormous increase in the size of the sinusoids following a short time after anastomosis, the diameter for a time decreases. Thereafter the diameter of the cylinders undergoes a steady increase at least to the time of birth or hatching.

14. The early increase in size of the cylinders is due to a multiplication of cells. The later increase is due to a growth in the size of the individual cells.

15 The hepatic nuclei which are originally large and elongately oval, become somewhat reduced in size and spherical in shape at the time when the tubules are formed from the tubule ridges. Thereafter they undergo a slow shrinkage in size up to the time of birth. In later stages they may again become oval or gibbus in outline, due to the pressure of the intracellular fat. The nuclei are at first basal in position and later become either central or peripheral.

16. Hepatic nuclei first have the common embryonic type of structure which they retain longer than do the nuclei of most tissue. The embryonic type of structure is lost first in the nuclei of the gall bladder and major hepatic ducts, next in the minor hepatic ducts, and finally in the hepatic cylinders.

17. Fat appears in the cylinders at an early stage and eventually fills almost the entire cell as in the typical fat cell.

18. Selachians in which the omphalo-mesenteric veins are early developed to large proportions have slender hepatic tubules which indent the walls of the vessels and anastomose at a very early stage.

B. Development of the minor ducts

1. Two forms of bile duct formation exist in the selachian liver, that of evagination of the liver pouch, and that of transformation of pre-existing cylinders into bile ducts. The first and most primitive is more active in the selachians than in higher vertebrates and forms the major ducts and the proximal parts of the minor ones. The distal parts of the minor ducts are formed by cylinder transformation.

2. Ducts are only differentiated from cylinders which lie in contact with branches of the omphalo-mesenteric veins. The side of the cylinder lying towards the vein is always the first to differentiate and in the smaller ducts is the only part to be differentiated from the true hepatic cells.

3. The cells of the bile duct epithelium are structurally farther removed from the primitive hepatic cell type than are the cells of the hepatic cylinders.

C. The hepatic mesenchyma

1. The hepatic mesenchyma is derived from the splanchnic mesothelium from two zones and at two periods. In both cases the mesenchymal proliferation is associated with marked irregularities of the mesothelium.

2. The first mesenchymal proliferation is ventral and is associated with the formation of mesothelial villi on the right side and irregular mesothelial growths about the omphalo-mesenteric vein on the left side. This proliferation forms the mesenchymal tissue about the gall bladder, cystic duct, main trunk of the omphalo-mesenteric vein, and to an indeterminable degree the interstitial mesenchymal tissue of the ventral part of the liver.

3. The second and dorsal proliferation is associated with the formation of mesothelial funnels and anastomosing mesothelial tubules which at one time occupy the dorsal fifth of the liver. These mesothelial invaginations break down into mesenchymal cords which in turn form the interstitial mesenchymal tissue of the dorsal part of the liver.

4. These two zones of mesenchymal proliferation correspond with the two zones of splanchnic mesenchymal proliferation of the trunk region of selachian embryos as described by H. E. Ziegler.

5. The mesothelial covering of the liver is at first made of columnar cells which later become squamous and thereafter apparently give rise to little or no mesenchymal tissue. The last areas from which the primitive form of mesothelium disappears are the tips of the lateral lobes and the dorsal margins of the lateral and median lobes.

6. Generally the splanchnic mesothelium becomes reduced at once from a columnar to a squamous cell layer upon contact with a hepatic tubule or cylinder.

7. The primitive embryonic type of nuclear structure is lost in the mesenchymal cells as they are proliferated from the mesothelium. No sharp line of demarcation could be drawn between endothelial and mesenchymal cells.

D. Hepatic sinusoids

1. Hepatic sinusoids arise in selachians by intercresence of the hepatic cylinders with the omphalo-mesenteric vein. This intercresence may be brought about either (a) by the growth of the rami of the omphalo-mesenteric vein about the cylinders as in Acanthias, or (b) by the invagination of the vessel wall by growing cylinders as in Mustelus and Torpedo.

2. In the first type there may be recognized three stages of sinusoid development: (a) The first in which the sinusoids are sparse and differ from capillaries only in their terminations at either side in veins; (b) The second stage in which the sinusoids are greatly enlarged constituting approximately 50 per cent of the liver; (c) The final stage in which the sinusoids take the form of the capillary sinusoids of Minot. In the second type of intercresence only the last two stages are present.

3. The reduction of the sinusoids from stage (b) to stage (c) is due to the growth in size of the hepatic cylinders and not due to their increase in number, for the most active increase in number of cylinders takes place prior to or early in stage (b).

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PLATE 1

EXPLANATION OF FIGURES

17 Reconstruction of the hepatic anlage of an Acanthias embryo 7.5 mm. long (S.C. 14) showing longitudinal tubule ridges upon the wall of the lateral hepatic pouch.

18 Reconstruction of the hepatic anlage of an Acanthias embryo 10 mm. long (S.C. 20) showing the formation of individual tubule anlagen from the tubule ridges.

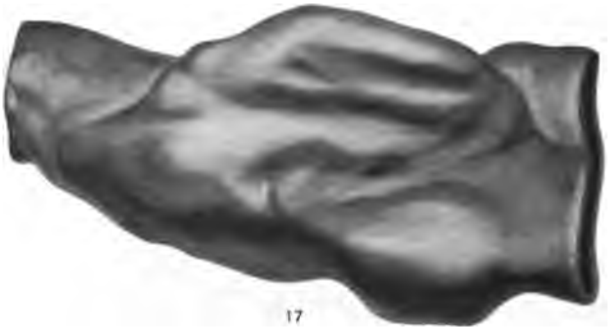
19 Reconstruction of a hepatic tubule of an Acanthias embryo 13.3 mm. long (S.C. 18) showing division into dorsal chamber and proximal neck and the secondary tubules arising from the former.

20 Reconstruction of a more highly developed tubule; from the same specimen as figure 19.

21 Reconstruction of two simple tubules from the lateral hepatic pouch of an Acanthias embryo 15 mm. long (H.E.C. 227).

22 and 23 Two views of a reconstruction of a highly developed hepatic tubule just prior to anastomosis; some of the secondary tubules are in contact; from the same specimen as figure 21.

24 Reconstruction of three anastomosing tubules; from the same specimen as figure 21; tubules *A* and *B* are completely fused; tubules *C* and *A* are in an early stages of the process.



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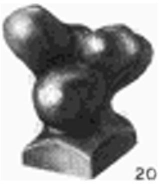

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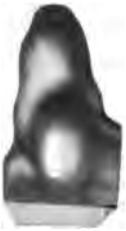

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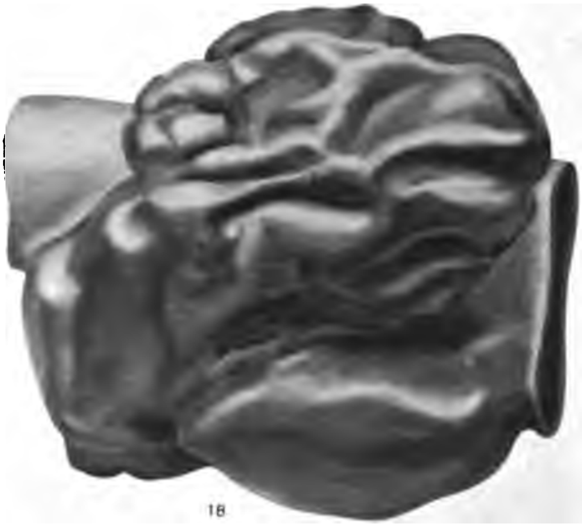

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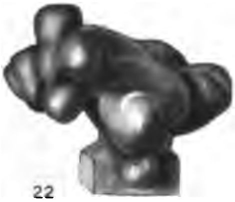

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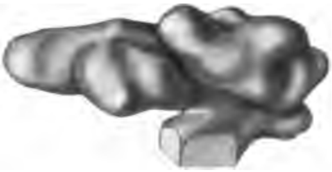

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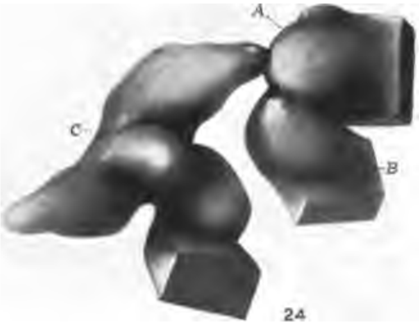

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PLATE 2

EXPLANATION OF FIGURES

25 to 27 Sections of hepatic tubules of Acanthias illustrating the process of anastomosis; (fig. 36 represents a step between figs. 26 and 27).

25 Early contact stage in anastomosis; from an embryo 13.3 mm. long (S.C. 18); alum hematoxylin. × 400.

26 Fusion of tubules; from an embryo 19 mm. long (S.C. 3); alum hematoxylin. × 400.

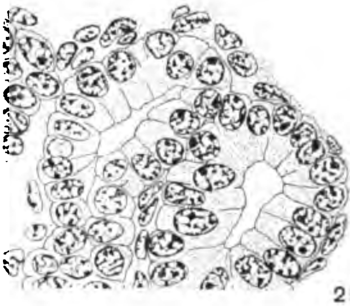
27 Establishment of connecting lumen between two anastomosed tubules. From an embryo 19 mm. long (S.C. 2); alum hematoxylin. × 400. Graphic reconstructions of these tubules, shown in cross section in figures 26 and 27, are illustrated in figure 2, page 224.

28 to 30 Sections of the external walls of lateral hepatic pouches of Acanthias embryos illustrating the formation of the hepatic tubules.

28 Formation of tubule ridges; from an embryo 8 mm. long (K.U.E.C. 545); iron hematoxylin. × 600.

29 Early outpouching of individual tubules. From an embryo 10 mm. long (S.C. 20); alum hematoxylin. × 400.

30 Completely formed tubules at the time when anastomoses first appear; from an embryo 13.3 mm. long (S.C. 18); alum hematoxylin. × 400.



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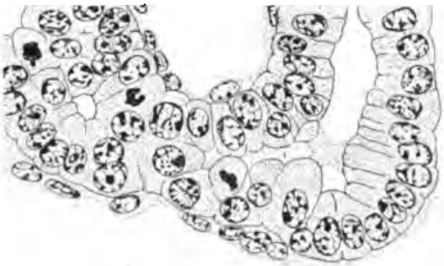

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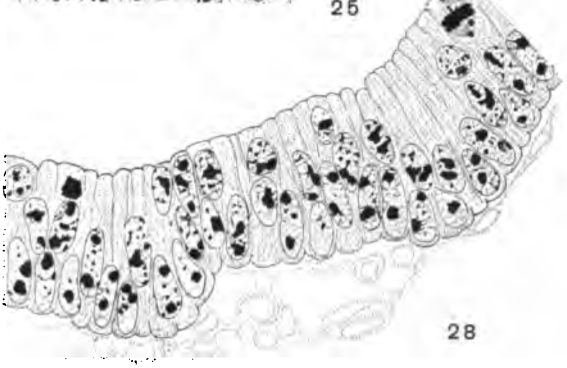

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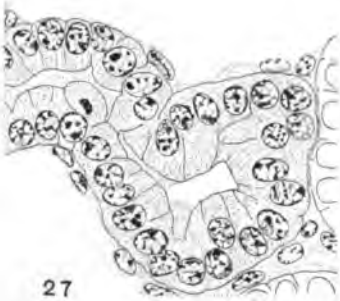

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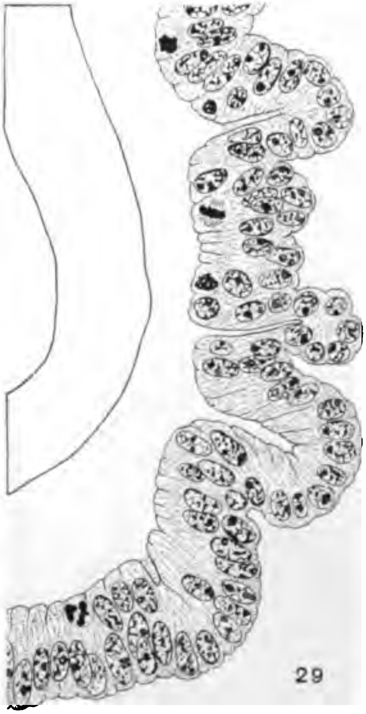

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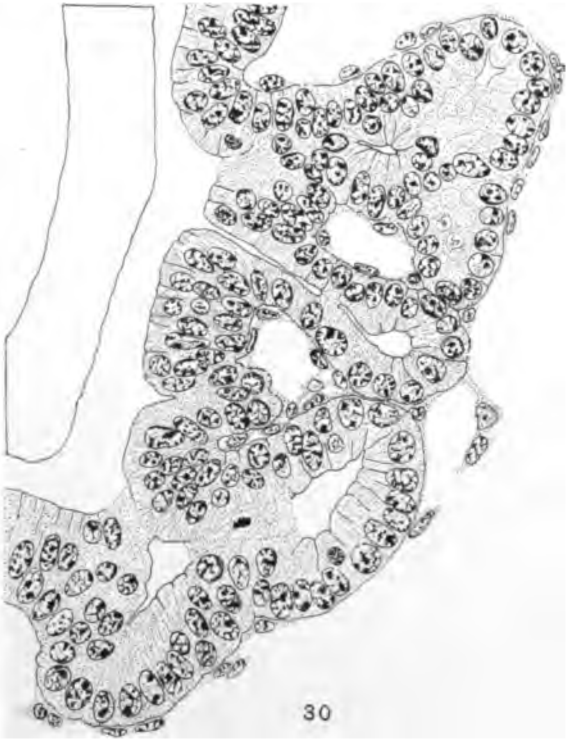

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PLATE 3

EXPLANATION OF FIGURES

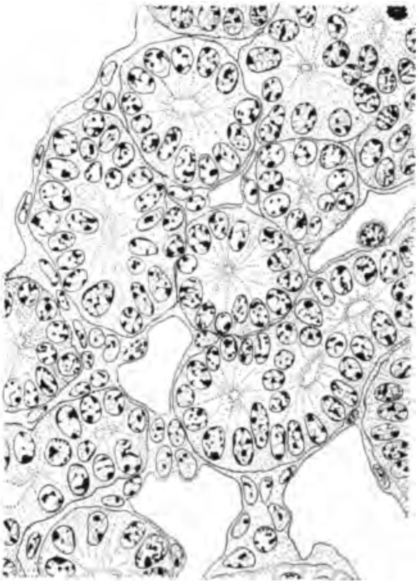
31 to 34 Four sections of livers of Acanthias embryos of different ages, illustrating the changes in the hepatic cylinders; all × 400.

31 Section of the liver of an embryo 16 mm. long (K.U.E.C. 547); iron hematoxylin.

32 Section of the liver of an embryo 20.6 mm. long (H.E.C. 1494); iron hematoxylin.

33 Section of the liver of an embryo 32 mm. long (H.E.C. 1652); iron hematoxylin.

34 Section of the liver of an embryo (of Squalus sucklii) 95 mm. long (H.E.C. 1882); alum cochineal.



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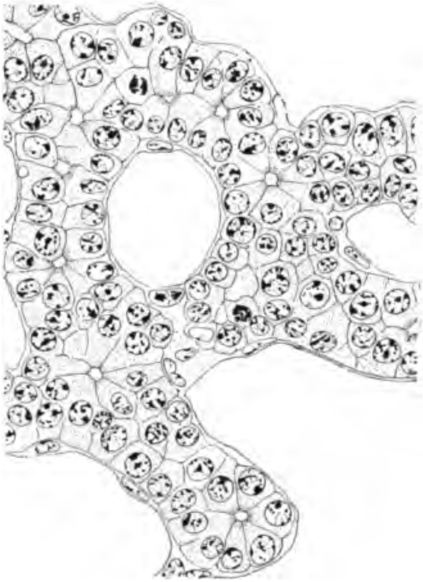

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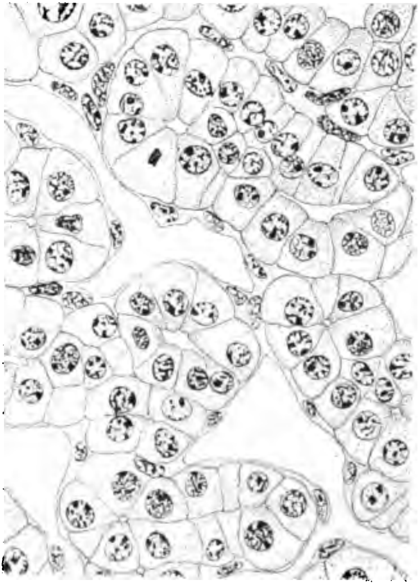

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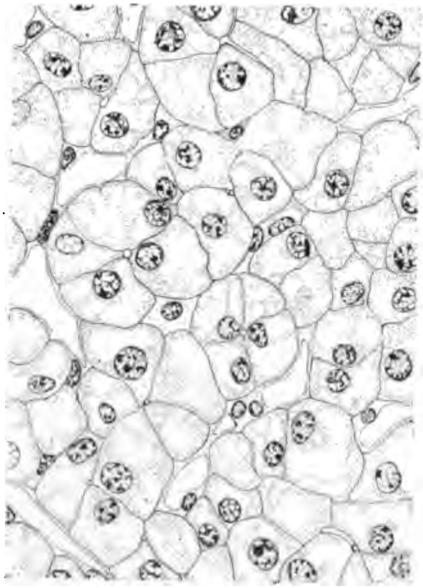

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PLATE 4

EXPLANATION OF FIGURES

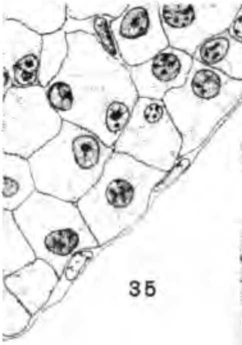
35 Transverse section of the margin of the liver of an embryo of Torpedo ocellata 29 mm. long; alum hematoxylin. × 350.

36 Section of an anastomosis between two hepatic tubules of an Acanthias embryo 14 mm. long (S.C. 30); alum hematoxylin. × 500. (This section shows a stage intermediate to those shown in figures 26 and 27).

37 Transverse section of a terminal bile duct of an embryo 95 mm. long (H.E.C. 1882); alum cochineal. × 400.

38 Hepatic tubules and mesenchymal ingrowths from a transverse section of the dorsal margin of the liver of an Acanthias embryo 24.7 mm. long (H.E.C. 1492); iron hematoxylin. × 400. *M*, mesenchymal ingrowths. *H.t.*, hepatic tubules.

39 Transverse section of a developing bile duct of an Acanthias embryo 24.7 mm. long (H.E.C. 1492); iron hematoxylin. × 400.



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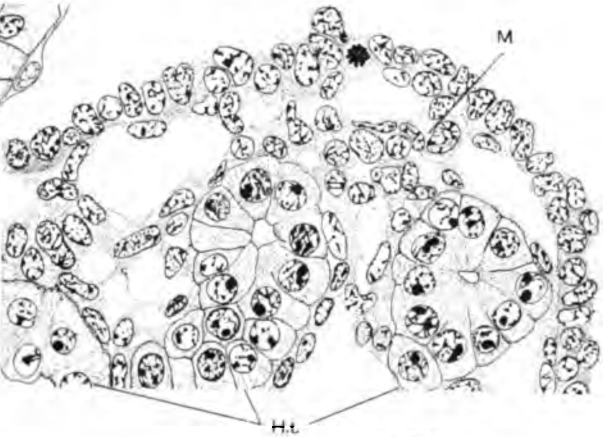

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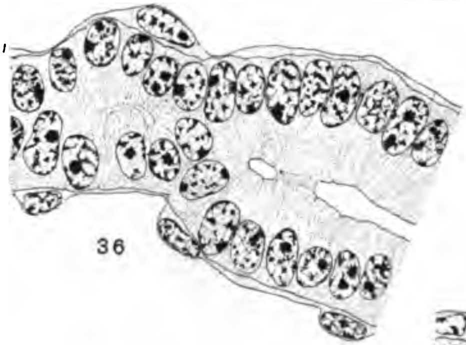

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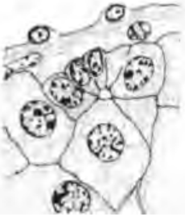

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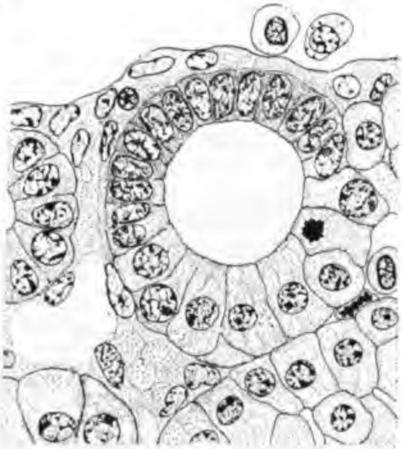

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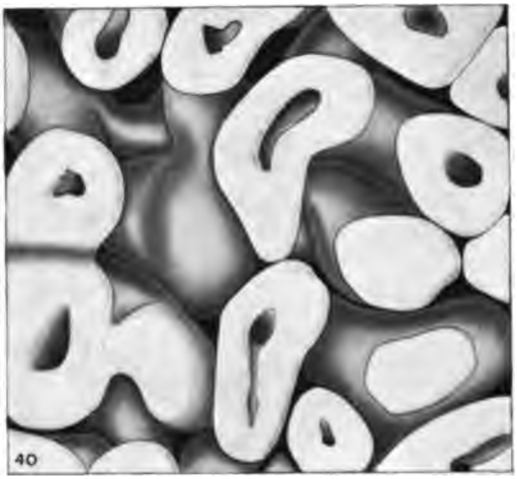
PLATE 5

EXPLANATION OF FIGURES

40 to 42 Portions of thick sections of livers of Acanthias embryos of different ages. The method of preparation is described in footnote 4, page 225. All approximately × 200.

40 Thick section of the liver of an embryo approximately 15 mm. long.
41 Thick section of the liver of an embryo approximately 21 mm. long.
42 Thick section of the liver of an embryo approximately 30 mm. long.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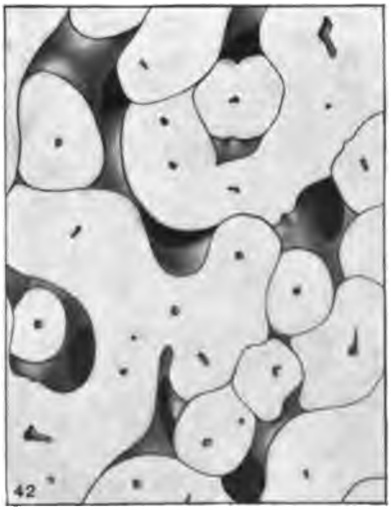
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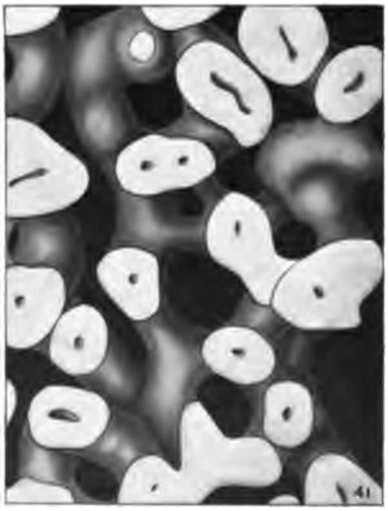
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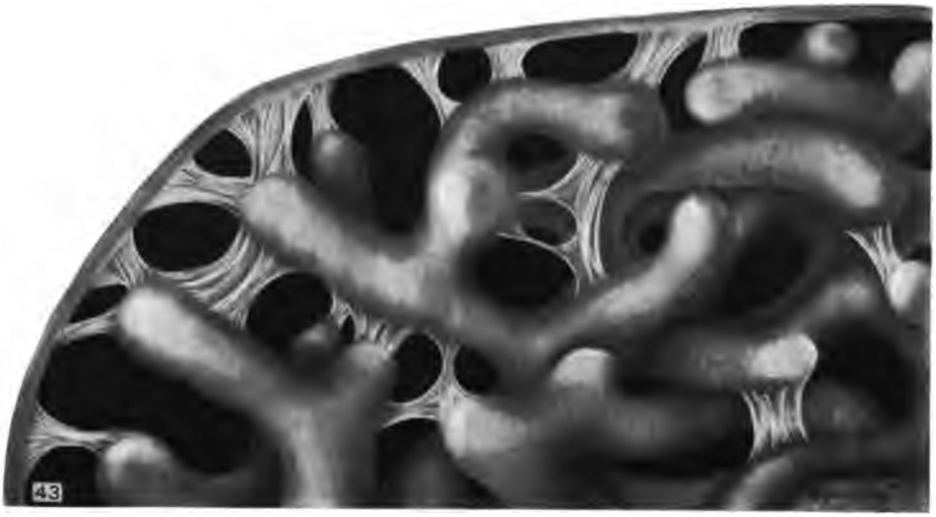
PLATE 6

EXPLANATION OF FIGURES

43 Preparation of the periphery of the posterior lobe of an Acanthias embryo approximately 20 mm. long showing the terminal buds of the hepatic network and the attachment of the network to the mesothelial covering by means of mesenchymal strands.

44 Thick frontal section of the liver of an embryo of Mustelus canis approximately 12 mm. long. The large hepatic duct and the hepatic tubules lie in a vascular sinus which is represented in black. The vertical band seen on the right hand side is a section of the mesothelial covering of the liver.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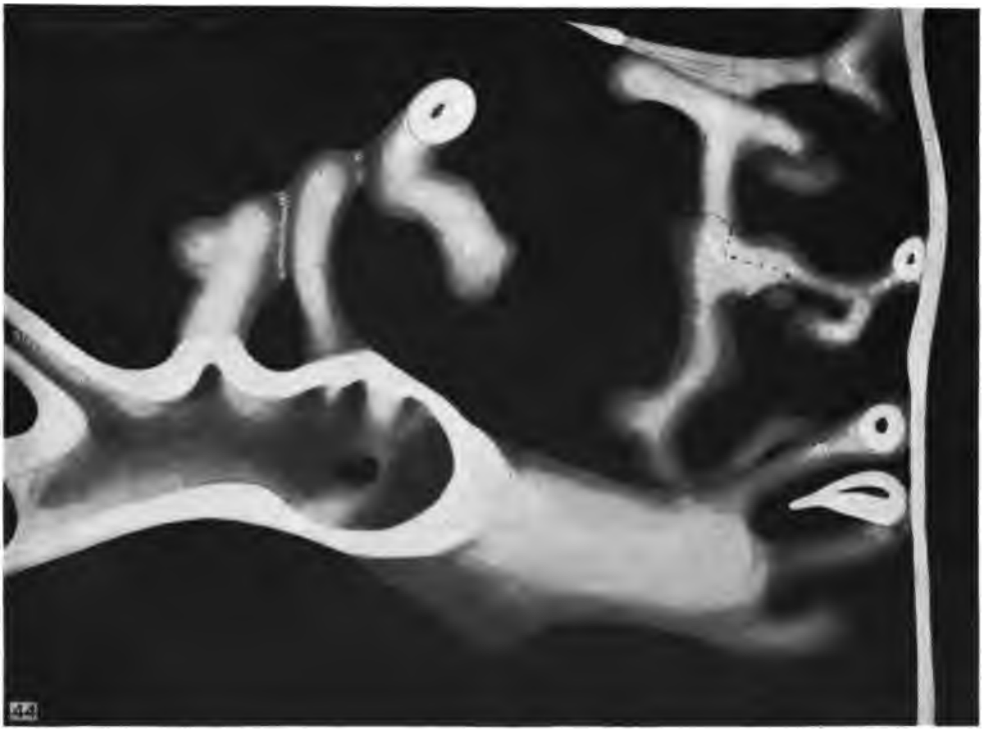
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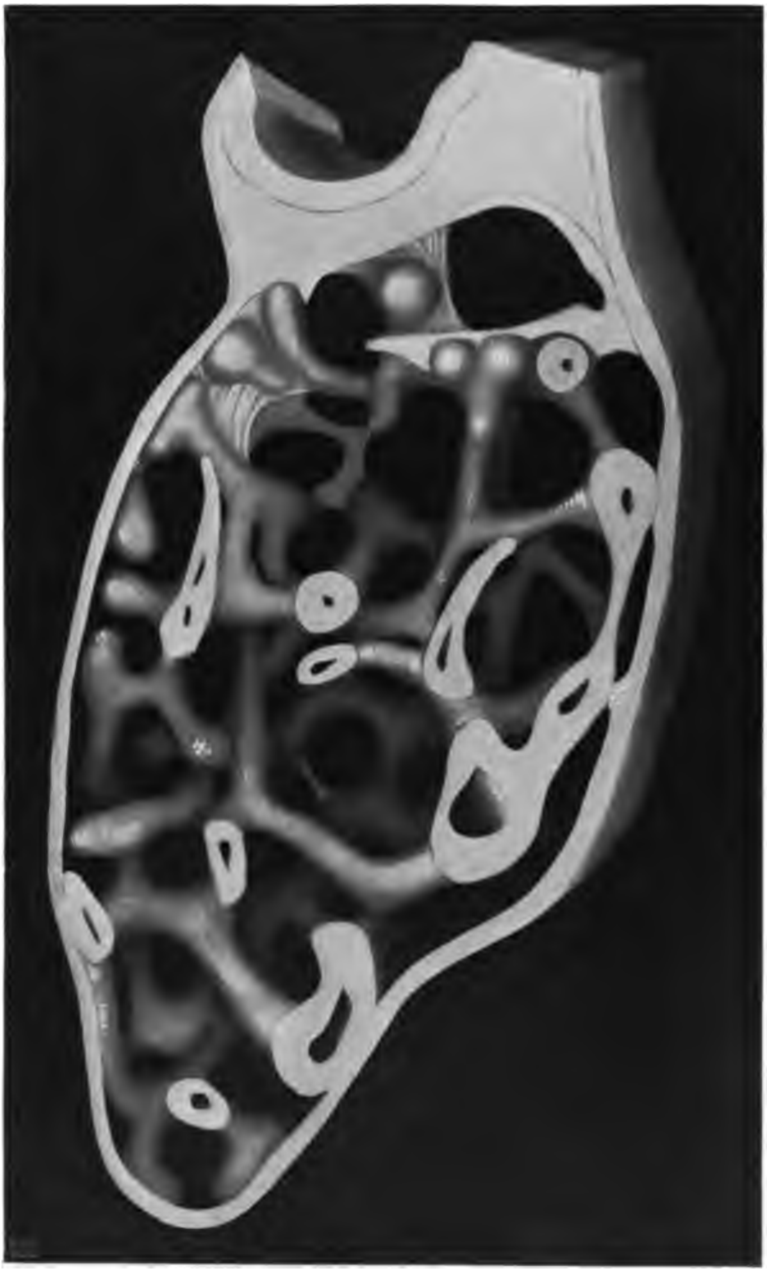
PLATE 7

EXPLANATION OF FIGURE

45 Thick frontal section of the liver of an embryo of Mustelus canis approximately 16 mm. long.



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